Distribution, occurrence, and identification of mosquito species in the Tongatapu Island Group, Kingdom of Tonga

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by

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In July 2011, Russell Taylor and EcoCARE Pacific Trust aided my first trip to Tonga for a volunteer teaching experience at Tonga High School. This was incredibly beneficial and stemmed my initial interest in the Kingdom of Tonga and mosquitoes. A few months later, I met Jon Harding for the first time when he was teaching a 2nd year Biology course (BIOL 273) and read his paper on the

'*Distribution and habitats of mosquito larvae in the Kingdom of Tonga*'. I was instantly captivated by mosquitoes and thought it would be very cool to return to Tonga to update this research. A few years passed since that time, and it wasn't until I enrolled in a Masters of Water Resource Management degree in 2013 that this dream became a reality. While other students were taking summer breaks from studies in 2013, I was searching for mosquito larvae in the Kingdom of Tonga! On this note, I would like to thank Jon Harding once again for funding the entire trip to Tonga, and to Meadow Mushrooms for providing me with a scholarship which ensured that I had enough money to live off when I returned to New Zealand.

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Preface

This thesis has been presented with the intention that each chapter, with the possible exception to the first and last chapters, will be submitted as peer reviewed journal articles. Scientific papers are the currency of contemporary scientists, therefore the publication of chapters from this thesis is important to myself – an aspiring scientist. This style promotes information dissemination, the primary goal of scientific research. With this in mind, there is some repetition of methodology in each chapter.

Abstract

Mosquitoes pose a serious threat to the economy, health status, and biosecurity of countries around the world. Mosquitoes kill an average of 700,000 people per year. The global expansion of air, sea, and land transport networks has greatly enhanced the spread of mosquitoes internationally. In the Pacific, the number of mosquito-borne diseases occurring has been on the rise in recent years, possibly as a result of human-mediated dispersal of larvae and adult mosquitoes. The Kingdom of Tonga has had numerous outbreaks of dengue fever and chikungunya virus in recent years. Previous research has catalogued species occurrences and distributions throughout Tonga. However, it is unknown whether new species have arrived in Tonga, and if distribution of previously found species has changed since the last comprehensive survey in 2006. Present research aims to update the literature by conducting a mosquito survey at 84 sites across the four islands of Tongatapu, Pangaimotu, ‘Oneata, and ‘Eua to record the distribution and occurrence of mosquito larvae. Nine mosquito species were collected: *Aedes aegypti* Linnaeus, *Ae. albopictus* Skuse, *Ae. tongae* Edwards, *Ae. horrescens* Edwards, *Ae. vexans nocturnus* Theobold, *Culex annulirostris* Skuse, *Cx. albinervis* Edwards, *Cx. quinquefasciatus* Say and *Cx. sitiens* Wiedemann. The collection of *Ae. albopictus* is the second time that this species has been recorded in Tonga. Moreover, the spatial extent of this species throughout Tonga was far greater than previously recorded. A major outcome of this survey has been the creation of an identification key for the mosquito larvae species of Tonga. This key should increase the accuracy of positive mosquito larvae identifications in Tonga. Mosquitoes were more frequently collected in artificial (e.g., used car tyres, fuel drums, containers) than natural (e.g., pools, ponds, tree holes) habitats. Car tyres, water containers, fuel drums, fridges, washing machines, and ponds were the most common habitats in which mosquito larvae were found. *Aedes aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* were the three most common mosquito species collected, whereas *Ae. tongae, Ae. horrescens, Ae. vexans nocturnus, Cx. annulirostris, Cx. sitiens*, and *Cx. albinervis* were less frequently found. Multiple logistic regression analyses indicated that habitat volume had a significant positive effect on the presence of *Ae. albopictus* and *Ae. tongae*,

whereas conductivity had a significant positive effect on the presence of *Cx. annulirostris*. Additionally, the volume by temperature interaction was a significant predictor of species presence for *Ae. aegypti*, *Ae. albopictus*, and *Cx. annulirostris* (as habitat volume increases, the effect of temperature went from neutral to negative). This suggests that larger, cooler habitats favour colonisation by these species. The number of artificial habitats (particularly used car tyres) present may have significantly increased since previous studies. Management should therefore focus on implementing community-run mosquito projects aimed at reducing the number of artificial habitats capable of being colonised by mosquito larvae. Covering, tipping out water, and infilling these habitats with soil to prevent mosquito oviposition is a pragmatic and straightforward mosquito control solution. This should immensely reduce the abundance of mosquitoes and help prevent disease outbreak in Tonga.

Frontispiece: Tongan children collecting mosquito larvae in vials for further identification using pipettes, turkey basters and a mosquito dipper from a tree hole in the village Matangaake, Tongatapu, December 2013.

Chapter One: General introduction to mosquitoes

General overview

Mosquitoes belong to the Phylum Arthropoda, Class Insecta, Order Diptera, and Family Culicidae. There are over 41 genera of mosquitoes, and approximately 3,500 described species, many of which are known vectors of human and zoonotic diseases (Spielman and D'Antonio 2001; Service 2008). *Anopheles, Aedes* and *Culex* are medically significant genera, due to their ability to transmit mosquito-borne diseases such as, malaria, dengue fever, and chikungunya virus.

Between the 17th and 20th centuries mosquitoes were responsible for the spread of more disease and human fatalities than anything else (Gubler 1998). In the late 19th century 350–500 million clinical cases of malaria resulted in approximately one million deaths each year (Tolle 2009). Similarly, dengue fever is responsible for 50–100 million infections annually and thousands of deaths are attributed to its more lethal form, dengue hemorrhagic fever (Tolle 2009).

Zoonotic mosquito-borne diseases can have detrimental effects on endemic fauna (Atkinson et al. 1995). The establishment of avian pox virus and malaria in Hawaiian forest bird populations has been attributed to the introduction of *Culex* mosquitoes in the early 19th century. This also has been linked with the decline in populations of the native Hawaiian honeycreepers (Atkinson et al. 1995).

The morphology, biology, and ecology of mosquitoes

Mosquitoes undergo complete metamorphosis; their life cycle involves four stages of development (Figure 1.1). Female mosquitoes lay eggs in a variety of artificial (e.g., used car tyres, fuel drums, containers) and natural (e.g., pools, ponds, tree holes) habitats (Figure 1.2). Species of *Aedes* and *Anopheles* lay eggs singly, whereas *Culex* species lay eggs in rafts of 200–300 eggs (Figure 1.3). Mosquitoes in the tribe *Aedini* lay waterproof eggs capable of resisting desiccation (Clements 2000).

The addition of water; including rainwater, flood water, and water added to containers by humans inundates mosquito habitats which stimulates the eggs to hatch into larvae.

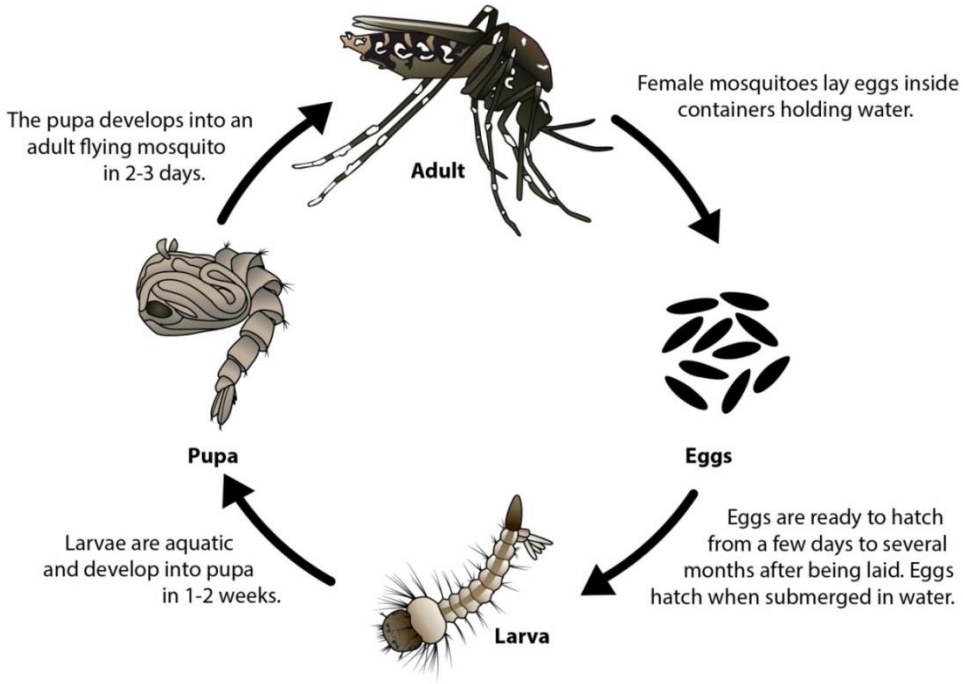

Figure 1.1: Mosquito lifecycle illustrating the four life stages (egg, larva, pupa, and adult). Figure modified from Centers for Disease Control and Prevention (2014).

Figure 1.2: Photomontage of mosquito habitats on Tongatapu, Kingdom of Tonga. This illustrates the variety of habitats in which mosquitoes may complete their larval and pupal lifestages. Images (a), (b), and (c) represent natural habitats (a pool, a pond, and car tyre tracks). Images (d), (e), and (f) represent artificial habitats (a freezer, an abandoned toilet, and a used car tyre) in which mosquito larvae were found.

The larvae, pupae and adults of *Aedes*, *Anopheles*, and *Culex* species all vary slightly in morphology (Figure 1.2). Larvae develop through four instars that shed their skin and increase in size at each moult. The main food resource of larvae is fine particulate matter; including bacteria, diatoms, algae, and detritus (Clements 2000). Larvae are predominantly filter-feeders, whose labral mouth brushes are used to generate a current of water in order to filter out suitable size particles for consumption and then pass these particles to the mouth. The larvae of some species can also feed by brushing submerged surfaces to dislodge organic matter which is then ingested (Clements 2000). Respiration occurs through the use of a posterior siphon to take in oxygen from the air at the water surface.

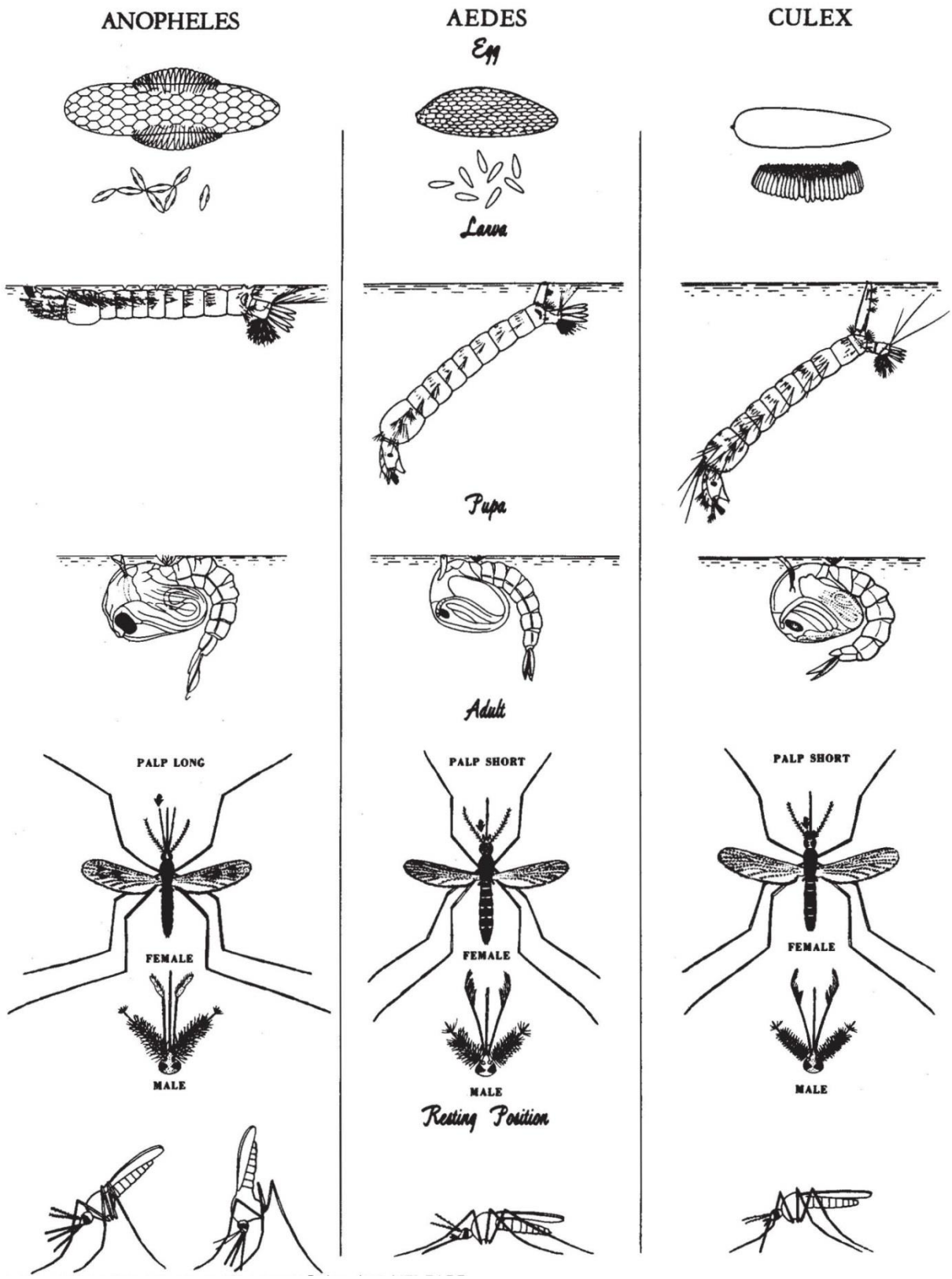

Figure 1.3: General characteristics of mosquitoes in the genera *Anopheles*, *Aedes*, and *Culex*. Modified from Littig and Stojanovich (2005).

Larvae in the fourth instar metamorphose into pupae. These pupae do not feed and float at the water surface, using their mesothoracic spiracles for respiration (Clements 2000). When disturbed they can move rapidly downward. When the adult is fully formed within the pupal cuticle, the insect sits at the water surface swallowing air. The consequent increase in internal pressure creates a split along the midline of the pupal cuticle, and enables the adult to "step" onto the water surface (Clements 2000).

Male mosquitoes are easily distinguished from females by their large and elaborate antennae (Figure 1.3). Males possess receptor organs that are attracted to the female flight tone (Belton 1994). Male mosquitoes are phytophagus, and are generally thought to feed on pollen and nectar obtained from the flowers. In contrast, female mosquitoes feed on pollen for energy, but also require a blood meal to provide protein needed for egg production (Clements 2000). Blood meals are typically obtained from mammals (e.g. cattle, horses, humans) or birds.

Carbon dioxide and body odour, carried by the wind, stimulate sense receptors on the palps and antennae of female mosquitoes which leads them to suitable hosts (Clements 2000). Blood is obtained by piercing the skin of a host with the sharp, modified mandibles and maxillae enclosed within the large, thick labium. The mouthparts enter peripheral blood vessels (Figure 1.4) and saliva is passed into them via the hypopharynx. The saliva contains a substance that prevents blood clotting and enables the female to feed continuously until abdominal stretch receptors signal repletion (Clements 2000). The food canal, up which blood is pumped by the mosquito, is formed between the hypopharynx and labium, which has a pair of lamellar lobes ventrally. Pseudo-tracheae within these lobes direct blood into the food canal.

Once an egg batch has matured, the female looks for a place to lay her eggs. Oviposition flight is strongly linked with environmental factors, of which rainfall, temperature, wind speed, and relative humidity are most important (Bentley and Day 1989). Visual, olfactory and tactile responses can be used by mosquitoes to determine the location and selection of oviposition sites (Bentley and

Day 1989). Light-dark contrasts, size and reflectance from water surfaces also influence oviposition site choice of some *Aedes* species (Harrington et al. 2008).

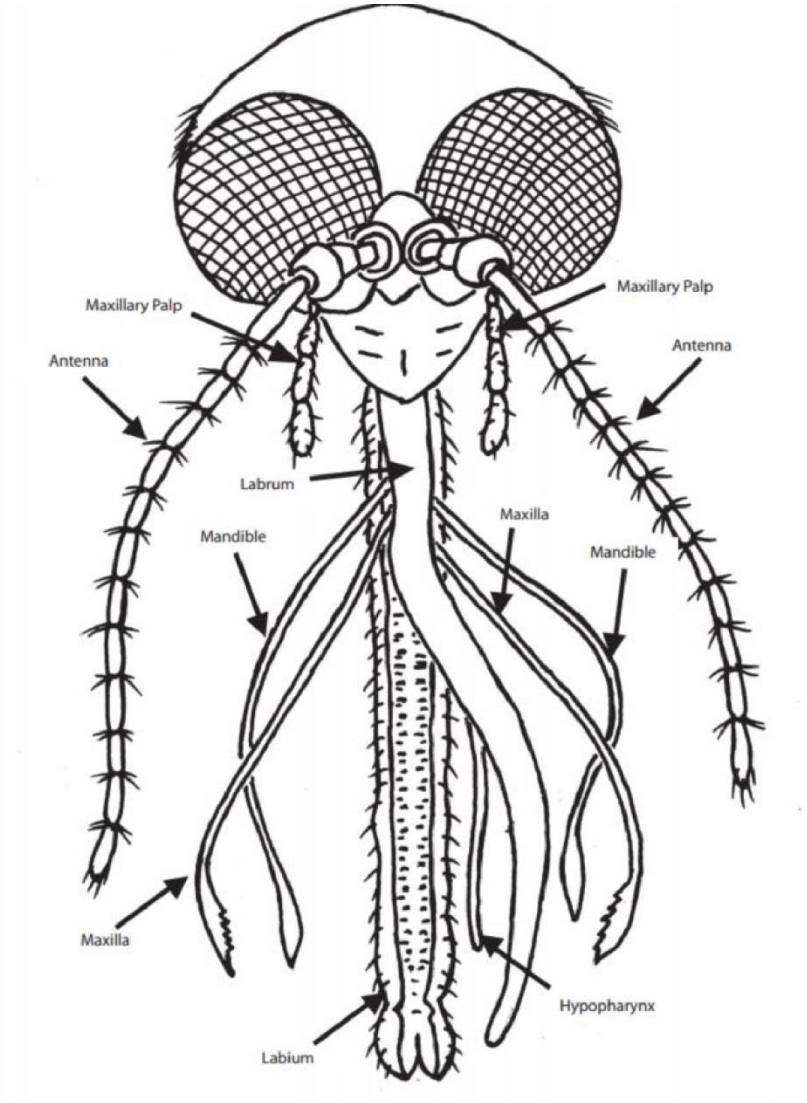

Figure 1.4: Mouthparts of a mosquito. Modified from Yale University, Peabody Museum of Natural History (2011).

Anthropogenic-mediated spread of mosquitoes and disease

The global spread and dispersion of mosquitoes poses threats to the economy, health status, and biosecurity of countries around the world (Service 2008). This has been enhanced by the substantial expansion of international air, sea, and land transport networks, and the international trade in used car tyres (Reiter 1998; Tatem et al. 2006). This expansion of transport has enabled geographically isolated ecosystems to become connected, and resulted in increased global movement of organisms and diseases (Lounibos 2002; Benedict et al. 2007). *Aedes albopictus* Skuse, a highly invasive mosquito originating in Asia, has dispersed to at least 28 other countries around the globe (Benedict et al. 2007). This spread is largely attributed to the international trade in used car tyres (Reiter and Sprenger 1987; Benedict et al. 2007).

Benedict et al. (2007) modelled the risk of invasion by *Ae. albopictus* throughout the globe by creating a Genetic Algorithm for Rule Set Prediction (GARP) used to predict and model the ecological niche of species. Mosquito occurrence data (presence/absence of mosquito species) and environmental data layers (e.g., annual mean temperature, annual mean precipitation, daily temperature range) were used in the model. Three risk factors were also included, these being; probable means of introduction via used car tyre shipments, shared borders with infested countries, and the extent of favourable area for establishment (Benedict et al. 2007). The model predicted a high areal distribution and occurrence of *Ae. albopictus* in South America (Figure 1.5) and throughout the Pacific (Figure 1.6). Suitable habitat, favourable climatic conditions, and the quantity of tyre importation were all strong predictors of mosquito distribution and occurrence.

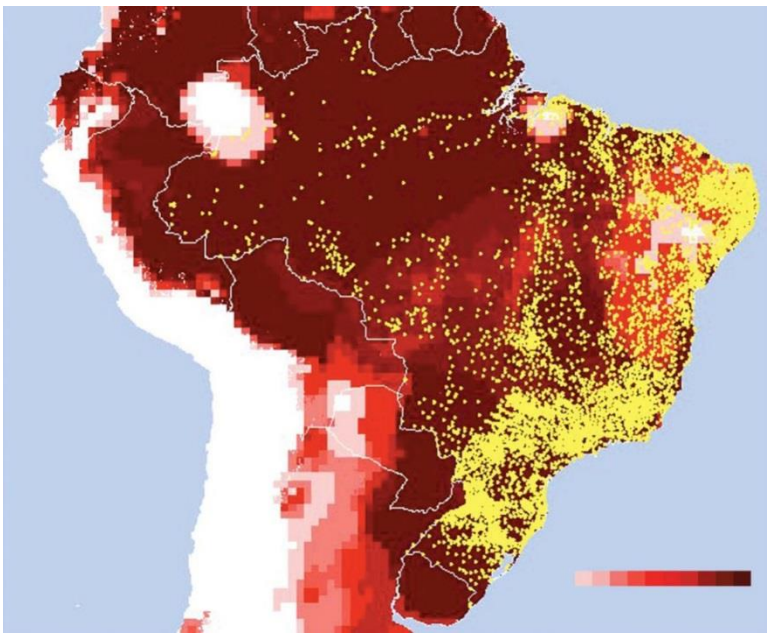

Figure 1.5: Predicted distribution (in red) and documented spread of *Ae. albopictus* (yellow points) in Brazil as of 2004. Darker shades of red indicate increasingly suitable mosquito habitat present. Figure modified from Benedict et al. (2007).

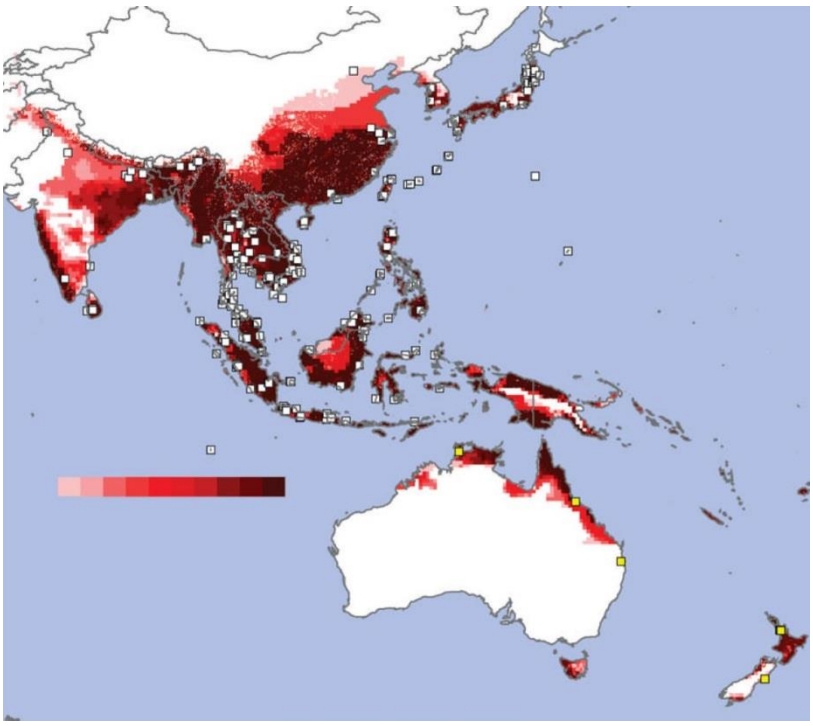

Figure 1.6: Predicted Asian and Australasian range of *Ae. albopictus*. Darker shades (signifying 10 models) indicate areas with the most potentially suitable niches. White squares represent the known occurrence points used to create predictive models. Yellow squares are known introduction sites outside the native range. Figure modified from Benedict et al. (2007).

The effects of climate change on incidence and distribution of mosquitoes

Rising atmospheric concentrations of greenhouse gases (e.g., carbon dioxide, methane, nitrous oxide), which have been mainly attributed to human activities (e.g., fossil fuel usage, land use change, agriculture) have likely caused the warming of the atmosphere and earth's surface (McMicheal et al. 2006). Published models on climate change presented by the Intergovernmental Panel on Climate Change (IPCC) predicted an increase in global temperature within the range 1.4–5.8°C for the year 2100 (Houghton et al. 2001). Projections of climate change indicate global warming as well as increased frequency and intensity of extreme weather events, such as, high and low temperatures, heavy rainfall, droughts, and floods (Easterling et al. 2000). Extreme climatic events (extreme high and low temperatures) are known to drive mosquito outbreaks by allowing more favourable conditions for mosquitoes to exist (Carrington et al. 2013; Chaves et al. 2014)

A predicted geographic consequence of climate change is that mosquitoes carrying malaria may extend their range into higher latitudes and altitudes, and mosquitoes carrying dengue fever may gain increased incidence and geographic range in the tropics (Reiter 2008). Reiters' (2001) earlier models suggested that increased temperature would allow mosquitoes to use previously cooler, unsuitable habitats. This may result in an increased abundance of mosquitoes, as well as increased disease transmission rates. Climate change, increased human activity, human impacts on local ecology, including mosquito breeding sites are therefore inter-related factors which may determine the prevalence and geographic range of disease-carrying mosquitoes in the future (Reiter and Sprenger 1987; Reiter 2001).

The spread of mosquitoes and diseases throughout the Pacific

Mosquito-borne diseases are an on-going threat for many countries around the Pacific (Hales et al. 1999). Invasive mosquitoes may have accompanied the Polynesians who colonised islands in the Pacific Ocean (LaPointe 2007), and voyages made by European explorers in the 15th century are a

likely cause of the initial spread of *Ae. aegypti* (Linnaeus) from West Africa to the Pacific (LaPointe 2007). Exploration and trade between the Old and New World tropics in the 17th to the 19th century likely enabled the spread of *Cx. quinquefasciatus* Say, and further increased the distribution of *Ae. aegypti* within the Pacific (LaPointe 2007). More recently, movements of troops and supplies during WWII and the Vietnam War almost certainly contributed to increased dispersal of mosquitoes in the Pacific. For example, 12 new mosquito species established in Guam following WWII and the Vietnam War (Ward 1984). These species included *Anopheles subpictus* Grassi an important vector of malaria and *Cx. tritaeniorhynchus* Giles a vector for Japanese encephalitis (Ward 1984). *Aedes albopictus*, a competent vector for at least 22 arboviruses was first reported in Papua New Guinea in 1970, the Solomon Islands in 1979 and Fiji in 1989 (Elliott 1980; Laille et al. 1990; Cooper et al. 1994). *Aedes albopictus* most likely dispersed as dormant eggs in car tyres (Gubler et al. 2001).

In recent years, an abundance of natural and artificial habitats and favourable climatic conditions (e.g., humidity, temperature) are likely to have mediated the spread of *Aedes* species in the Pacific (Figure 1.7) (Horwood et al. 2013). Notably, the presence of *Ae. aegypti* and *Ae. albopictus* may likely have given rise to a suite of mosquito-borne diseases in countries where these diseases had not previously been present. One such disease, chikungunya virus, has traditionally been absent from all countries within the Pacific (Horwood et al. 2013). However, recent outbreaks in Reunion Island (2005-06), New Caledonia (2011), and Papua New Guinea (2012) have raised significant concerns about its possible further spread (Reiter et al. 2006; Horwood et al. 2013). Similarly, in recent years dengue fever outbreaks have been more commonly reported in the Solomon Islands, Rarotonga, New Caledonia, Tonga, Samoa, Fiji, Cook Islands, and Hawaii (Centers for Disease Control and Prevention Dengue World Map 2013).

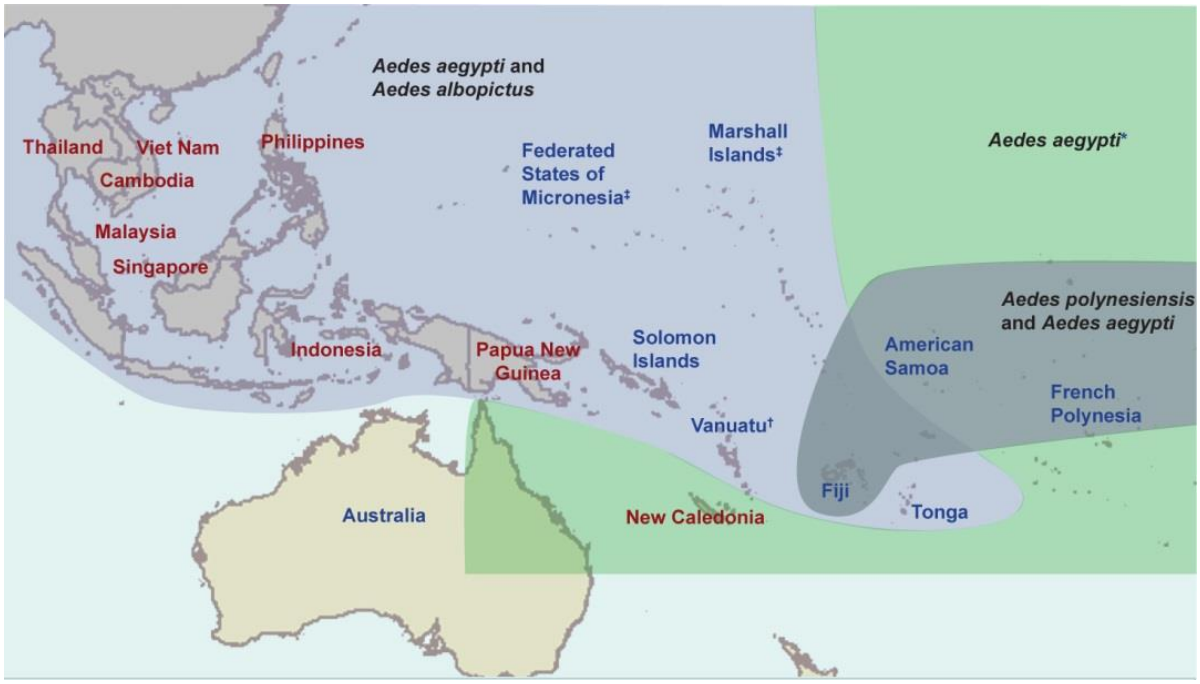

Figure 1.7: Distribution of *Aedes* species within the Pacific. Countries with names in red have had previous chikungunya outbreaks, whereas countries in blue have not. Modified from Horwood et al. (2013).

Tonga

The Kingdom of Tonga comprises a chain of over 170 islands located between Fiji, and Niue in the South Pacific Ocean between latitudes 15° and 23°30'S and longitudes 173° and 177°W (Figure 1.8) (Thompson 1986). Vava'u (96 km^2), Ha'apai (47 km^2) and Tongatapu (261 km^2) are the three main uplifted limestone island groups, and lie in a roughly north-south row (Fall and Drezner 2011). The Tongan islands formed as part of the forearc belt of the Tongan-Kermadec Trench system some time between about 1 and 10 million years ago (Dickinson 2001). Numerous submarine hydrothermal vents are commonplace throughout the Tongan arc (Stoffers et al. 2006).

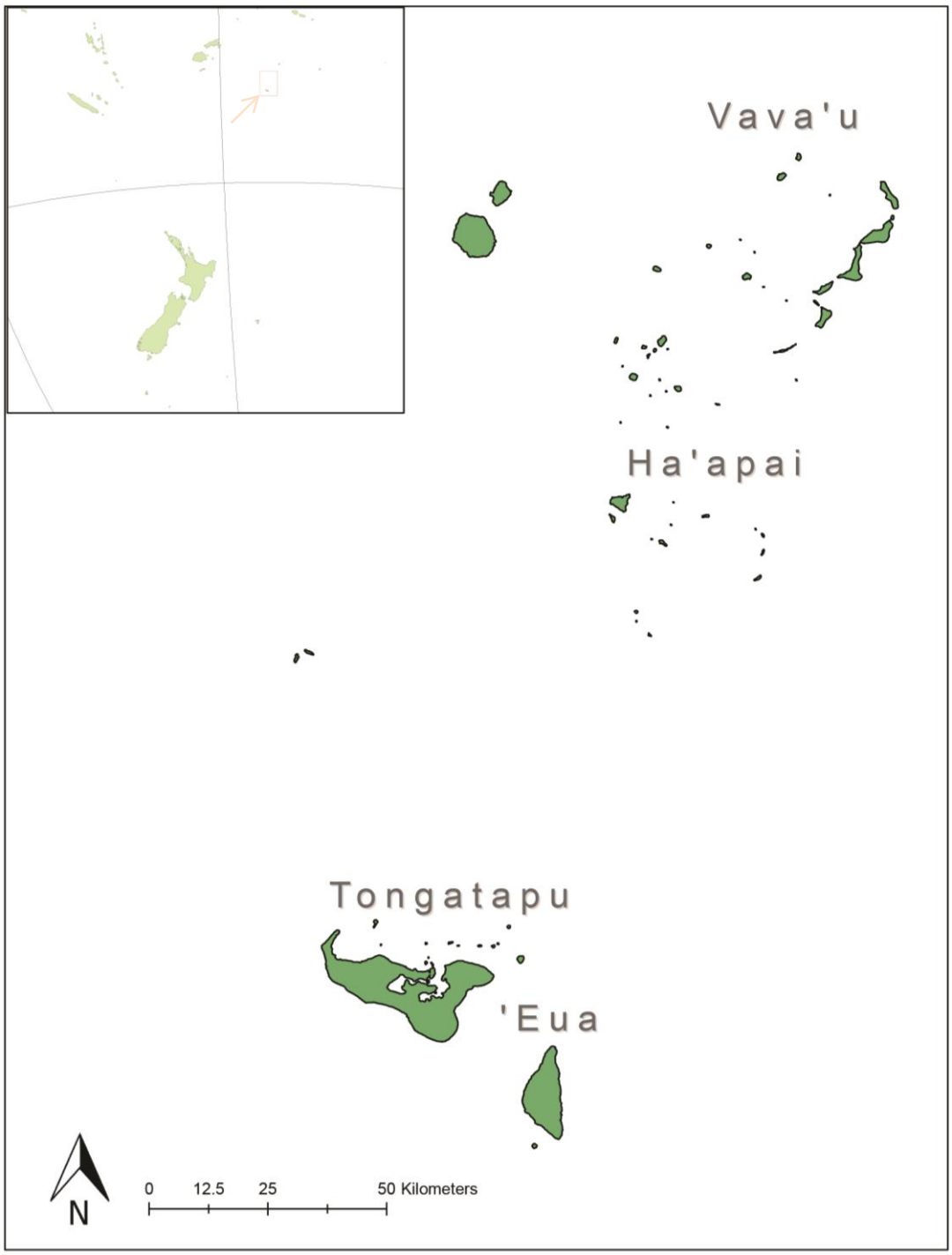

Figure 1.8: The major Islands making up the Kingdom of Tonga and their location with reference to New Zealand (insert). Tongatapu and ‘Eua islands are considered as the Tongatapu Island Group.

In 2010 Tonga had a population of 103,252 people (Tonga Department of Statistics 2011). Tongatapu is the most populous island, containing the capital city Nuku'alofa. All three islands have low-lying topography with little hilly terrain. Lake Tu'anuku in the Vava'u island group is one of the largest of the few water bodies in the country. Lotic water bodies within Tonga are almost completely lacking, with the exception of a few streams on 'Eua Island.

Tongatapu, with 75,000 inhabitants has a population density of 290 people/km^2, far more than on the smaller offshore islands of Vava'u (123 people/km^2), Ha'apai (61 people/km^2), and 'Eua (57 people/km^2) (Tonga Department of Statistics 2011). Life expectancy of Tongan women is 72 years, and that of men is 70 years (WHO 2015). Cardiovascular diseases are the most common causes of mortality, followed by neoplasmic, respiratory, infectious, and parasitic diseases (WHO 2015). One-storeyed, five bedroom houses are the most common form of accommodation in Tonga, and often house several generations of one family (Figure 1.9). Discarded car tyres and rubbish are commonly found in many properties, and provide excellent habitats for mosquito larvae (Figure 1.9). Most households do not have access to clean drinking water, and rely on rain water to fill concrete water tanks for this purpose. Many of these water tanks are not covered providing perfect refuge for mosquito larvae (Figure 1.9).

Tonga has a tropical climate with warm temperatures and high rainfall. Average annual temperatures range from 21–23.5°C (Thompson 1986; Stanley 1999). The dry season is May–October and the wet season is November–April. Annual precipitation ranges from 1,780–2,340mm (Thompson, 1986). The Tongan islands are positioned in the path of tropical cyclones and on average two strike the islands each year (Franklin et al. 2004).

Figure 1.9: Housing and common mosquito habitats surrounding houses. (a) A typical house and family in Tonga; (b) and (c) property rubbish and discarded car tyres in which mosquito larvae inhabit; (d) a concrete container used to store drinking water, which also contained mosquito larvae. Images (a) and (b) were captured by Stan Swan in 2013, (c) and (d) were captured by me.

Tonga is widely recognised as having a significant mosquito problem, from both a nuisance and medical perspective (Stanley 1999). The most recent survey in 2006 collected eight mosquito species, with an additional species collected in 2012 (Harding et al. 2007; Guillaumot et al. 2012). Some of these species collected have been responsible for outbreaks of mosquito-borne diseases in the Kingdom; namely dengue fever and chikungunya virus. Outbreaks of dengue fever have occurred in Tonga in 1974, 1975, 1998 and 2003 and have caused numerous fatalities (World Health Organization 2006). In 2014 and 2015, dengue fever outbreaks occurred sporadically every few months (Centers for Disease Control and Prevention 2012) and a widespread chikungunya virus outbreak affected 10,000 people in April 2014 (ABC news 2014). Public awareness programmes have been conducted by the Ministry of Health, World Health Organization and the Tongan Red Cross

Society to reduce incidence of the disease, and pesticide spraying for mosquito adults has been undertaken with variable success (Tonga Daily News, 2015).

Thesis organisation

The primary aim of my research was to re-assess the distribution of mosquito species in the Tongatapu Island Group and determine species distributions since the last major survey in 2006. Accordingly in Chapter 2 I present an identification key to mosquito larvae, based on the species found in my survey. In Chapter 3 I focus on the distribution of mosquito species in 2006, compared to those found in 2013. In Chapter 4 I investigate the occurrence of mosquito species with regard to abiotic factors and habitat selection. Finally in Chapter 5, a synthesis and general discussion is presented.

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Chapter Two: Identification key to Tongan mosquito larvae (Diptera : Culicidae)

Abstract

Currently, no accurate and up-to-date identification keys of mosquito larvae species existing in Tonga occur. Keys are provided for the identification of larvae of the nine mosquito species known to occur in the Tongatapu Island group, Kingdom of Tonga. Five *Aedes* species (*Ae. aegypti* Linnaeus, *Ae. albopictus* Skuse, *Ae. horrescens* Edwards, *Ae. tongae* Edwards, and *Ae. vexans nocturnus* Theobald) and four *Culex* species (*Cx. quinquefasciatus* Say, *Cx. annulirostris* Skuse, *Cx. albinervis* Edwards, and *Cx. sitiens* Wiedemann) were found in the 2013 survey of the Tongatapu Island group. The keys include microscope images and illustrations to show diagnostic morphological characters of third and fourth instar larvae.

Introduction

Aedes and *Culex* mosquitoes include species that are possible vectors of numerous mosquito-borne diseases which infect humans (Guillaumot 2005; Service 2008). The incidence of mosquito-borne diseases is increasing worldwide, partly due to increased airline travel and population mobility (Jones et al. 2008; Tatem et al. 2012). Successful colonisation of islands in the Pacific by virulent mosquito species has resulted in concurrent epidemic outbreaks of dengue, chikungunya, and zika virus infections in recent years (Roth et al. 2014). If not managed effectively, these epidemics may have widespread effects on societies. Tourism, health, trade, and work productivity could all be affected (as cited in Roth et al. 2014). During the chikungunya outbreak on Reunion Island in 2006, 33% of the 800,000 inhabitants were infected, with 47,000 estimated cases in the most severe week of the outbreak. This outbreak resulted in a loss of approximately €17.4 million worth of work

productivity, and the associated medical costs as a result of the disease totalled €43.9 million (as cited in Roth et al. 2014).

In order to assist health authorities and researchers to identify the larvae of mosquito species I have prepared an identification key of currently recorded taxa. Most taxonomic keys used to identify mosquito larvae are based on morphological characters (e.g. Belkin 1962; Huang 1977), but they can often be overly complicated for use by non-entomologists. However, correct species identification is important as it provides the user with the ability to access literature about the distribution, medical importance, ecology, and behaviour of the particular species. Currently, there is no complete and up-to-date key to the larvae of mosquitoes found in Tonga; workers have mainly relied on the descriptions and keys in Belkin (1962) and Rueda (2004). Consequently, it is likely that some larvae have been misidentified.

In this chapter I present, the main characters useful for identification of third and fouth instar larvae of five *Aedes* species: *Ae. aegypti* Linnaeus, *Ae. albopictus* Skuse, *Ae. horrescens* Edwards, *Ae. tongae* Edwards, *Ae. vexans nocturnus* Theobald and four *Culex* species: *Cx. quinquefasciatus* Say, *Cx. annulirostris* Skuse, *Cx. albinervis* Edwards, *Cx. sitiens* Wiedemann are described and illustrated.

Materials and Methods

Larval specimens used in this study were collected from 84 sites in the Tongatapu Island group in November–December 2013. They were stored in 50 ml vials of 70% ethanol and transported to the University of Canterbury, New Zealand for identification.

Larvae were identified using a Leica MZ125 Stereomicroscope with a 1.6x Leica Plan apochromatic lens (Leica 10446231). A Zeiss AxioCam HRC camera was attached to the housing of the microscope to acquire images and Zeiss AxioVision software (v4.5) was used to include a scale

bar on images. Images were saved in JPEG (Joint Photographic Experts Group) and TIFF (Tagged Image File Format) formats, cropped and finalised in Adobe Photoshop CC (64 bit).

In preparation for examination, larvae were moved from vials into a petri dish using a pipette and submerged in ethanol. Forceps were used to manipulate the specimen for optimum viewing under the microscope.

Magnification of 80–100x (10x eyepiece, 8–10x objective) was required to see morphological differences in comb scales of *Aedes* species, and 32–100x magnification (10x eyepiece, 3.2–10x objective) was required to see morphological differences in siphon hairs and gills of *Culex* species.

The terminology of Harbach and Knight (1980, 1982) is used to describe features of mosquito larvae. Entomological information about each species was obtained from Belkin (1962), Huang (1977), Huang and Hitchcock (1980), Ramalingam (1976), and Rueda (2004). The classification of species in the tribe Aedini follows that of Knight and Stone (1977). Classification of species follows Harbach (2014). Appendix figures were hand drawn by Mark Galatowitsch, and are based on drawings by Belkin (1962) and Rueda (2004).

Results

Morphological features referred to are shown in Figure 2.1. Key distinguishing features of the genera and species are used in a dichotomous key below. For larvae, these focus on the posterior segments and features particularly the siphon, saddle, and comb scales.

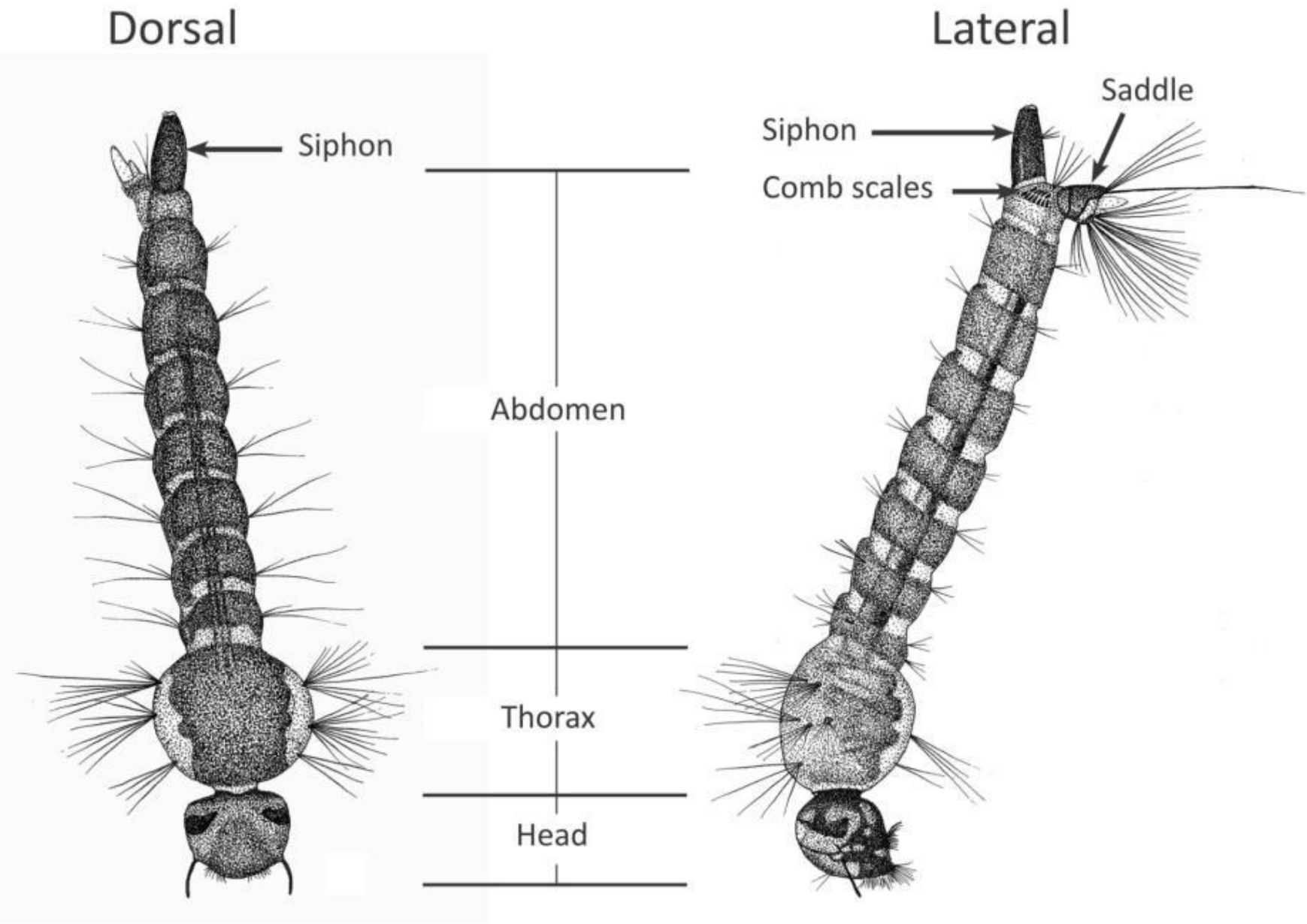

Figure 2.1: Morphological features of mosquito larvae referred to in the key.

Key to genera and species

1. Siphon about twice as long as wide (Fig. 2.2); with a row of 5-10 comb scales, either in a straight or slightly curved line (Fig. 2.3) ……*Aedes, 2*

   Siphon at least three times as long as wide (Fig. 2.4); with a cluster of 20-40 comb scales (Fig.2.5) ……*Culex, 6*

2. Comb scales with about 5 subapical spines, one being much longer and thicker than the others (Figs 2.6a, 2.6b, Appendix 2.1) ……*Ae. aegypti*

   - Comb scales without subapical spines (Fig. 2.7) ……3

3. Saddle of anal segment incomplete with a colourless, smooth patch ventrally interrupted between its ends (Fig. 2.8); about 8 comb scales in a straight line, the scales narrowing abruptly at about mid-length (Fig. 2.7, Appendix 2.2) ……*Ae. albopictus*

   - Saddle of anal segment not interrupted by a smooth surface (Fig. 2.9); comb scales not narrowing abruptly at about mid-length ……4

4. With 8-10 comb scales in a jagged, roughly V-shaped row (Fig. 2.10); anal gills narrow and pointed (Appendix 2.3) ……*Ae. vexans nocturnus*

   - Comb scales aligned in a slightly curved row (Fig. 2.11) ……5

5. With 8-14 narrow, sharp pointed comb scales (Fig. 2.11); anal gills not much longer than anal segment, their tips rounded (Appendix 2.4) ……*Ae. horrescens*

   - With 8-14 narrow, sharp pointed comb scales (Fig. 2.12); anal gills 2-3 times longer than anal segment, their tips rounded (Appendix 2.5) ……*Ae. tongae*

6. Siphon with 3 pairs of subventral hairs; siphon narrow, its length about 9 times mid-width (Fig. 2.13, Appendix 2.6) ……*Cx. albinervis*

   - Siphon with more than 3 tufts of subventral hairs ……7

7. Siphon with 4 tufts of subventral hairs; siphon length about 3.5 times its mid-width (Fig. 2.14, Appendix 2.7) ……*Cx. quinquefasciatus*

   - Siphon with 5-7 tufts of subventral hairs ……8

8. Siphon with with 5-7 tufts of long subventral hairs; Siphon length 5-7 times its mid-width (Fig. 2.15); anal gills longer than anal segment, their tips rounded (Appendix 2.8) ……*Cx. annulirostris*

   - Siphon with 5-6 subventral tufts of hair; Narrow siphon length 7-8 times its mid-width (Fig. 2.16) 4 anal gills shorter than anal segment, each gill tapering to a point (Fig. 2.17, Appendix 2.9) ……*Cx. sitiens*

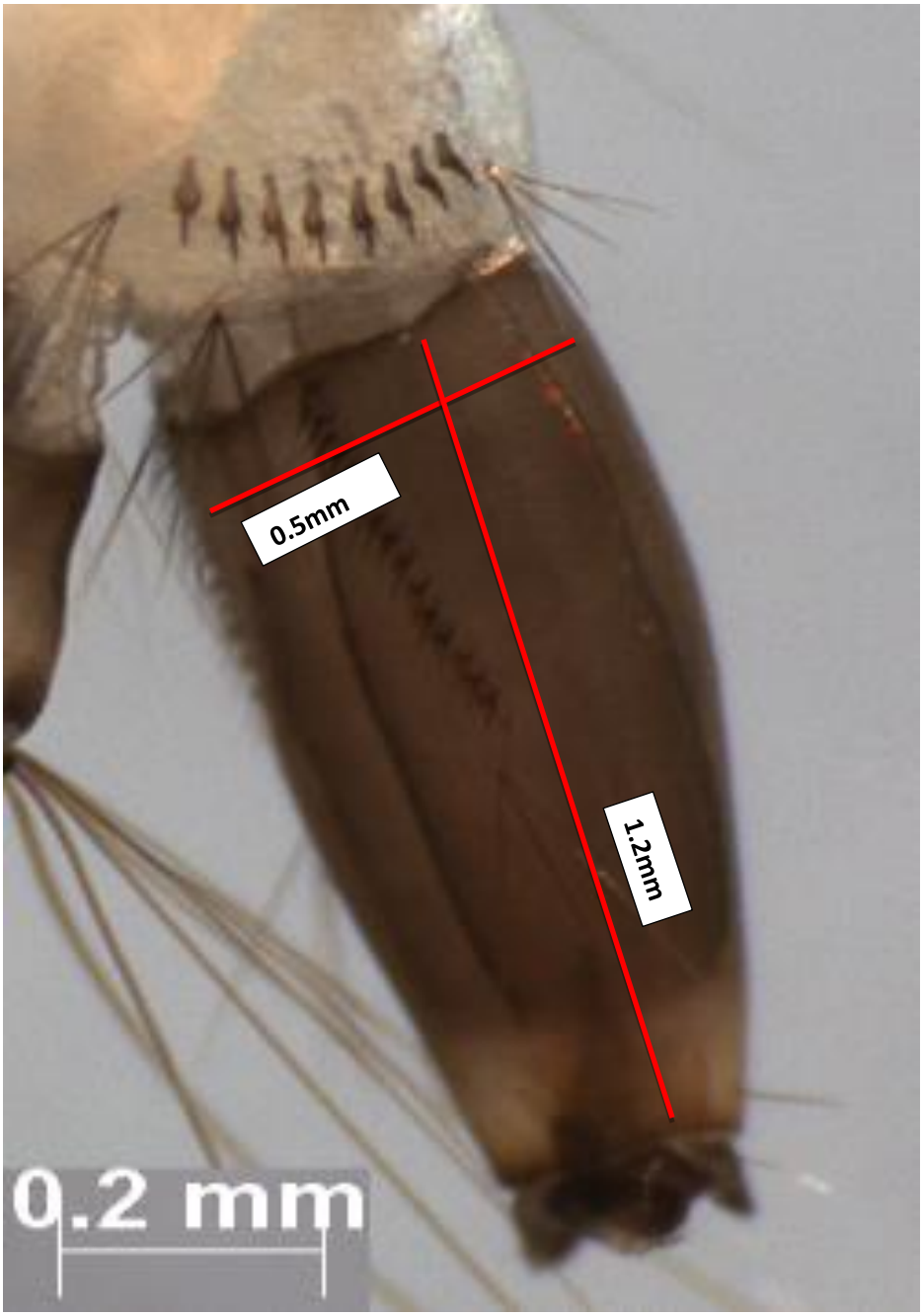

Figure 2.2: Siphon about twice as long as wide in *Aedes* (Animal shown is *Ae. aegypti*).

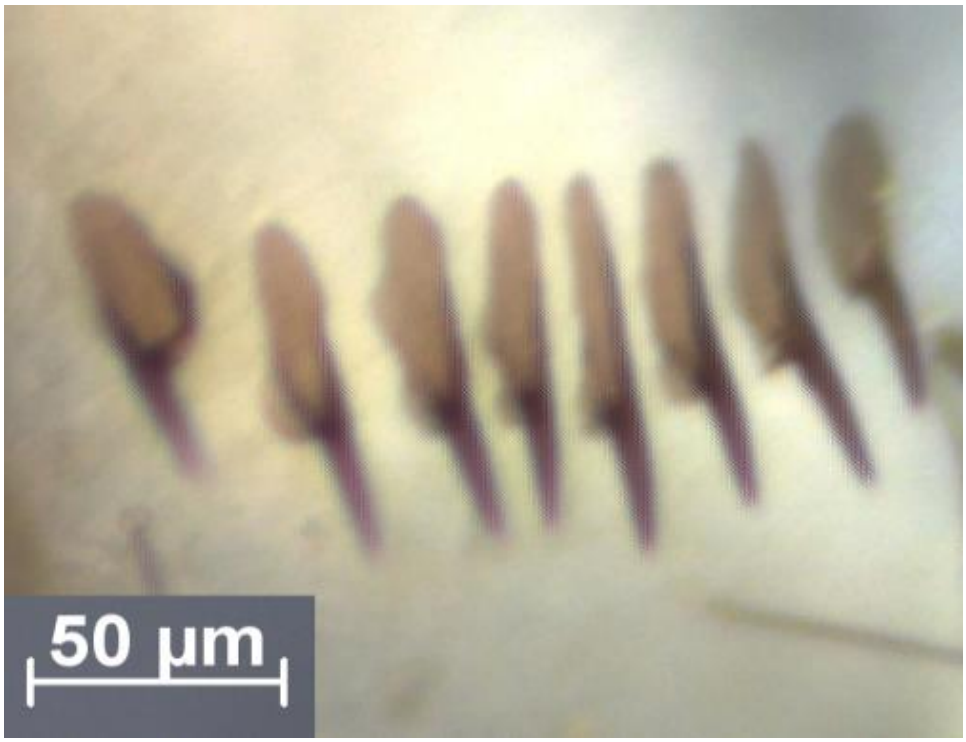

Figure 2.3: *Aedes* with 5-10 comb scales. (Animal shown is *Ae. albopictus*).

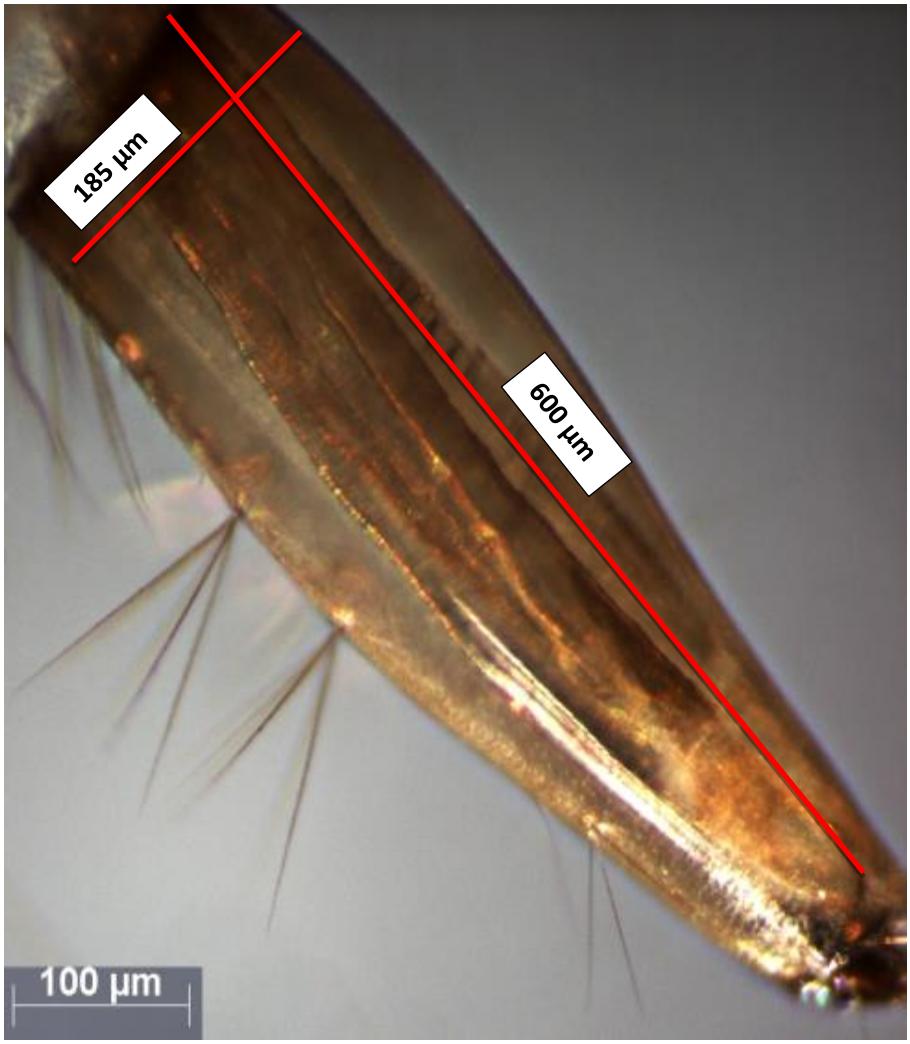

Figure 2.4: Siphon of *Culex* at least three times as long as wide. (Animal shown is *Cx. quinquefasciatus).*

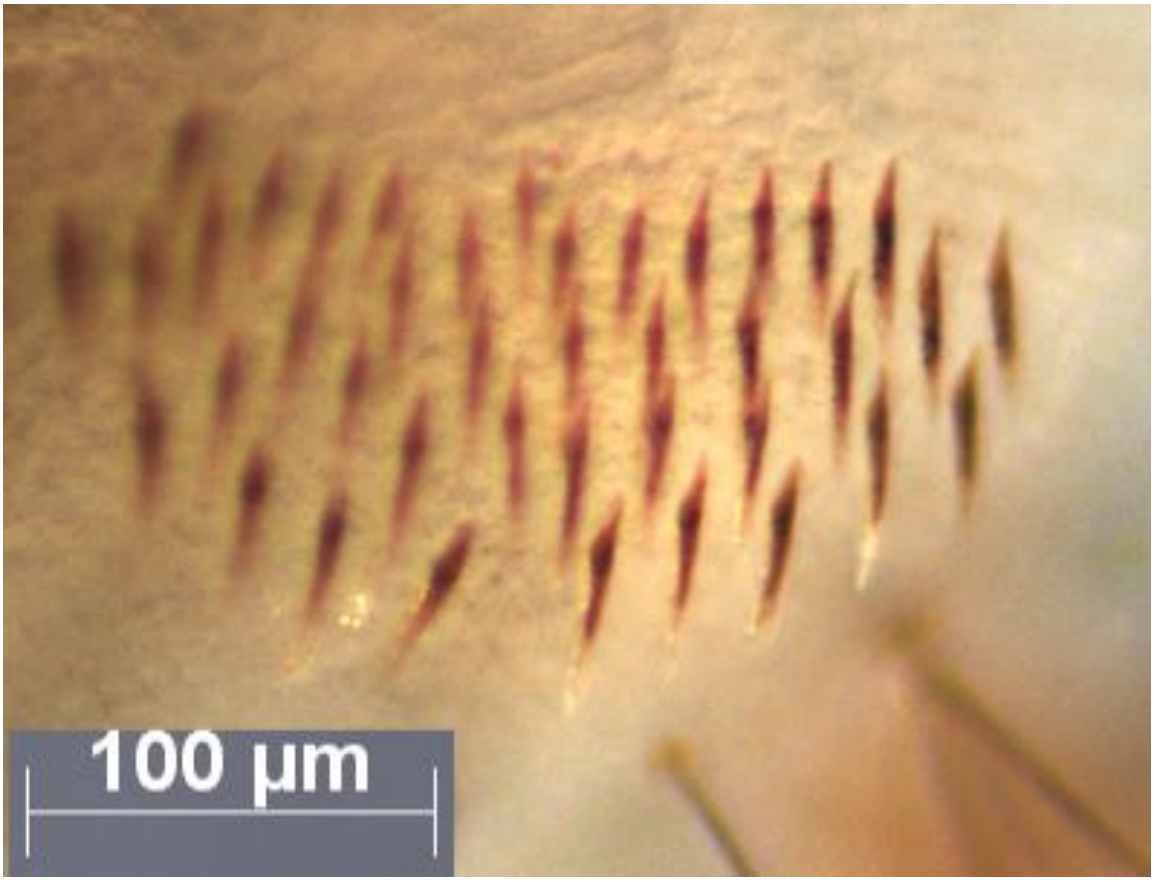

Figure 2.5: *Culex* with 20-40 comb scales in cluster. (Animal shown is *Cx. sitiens*).

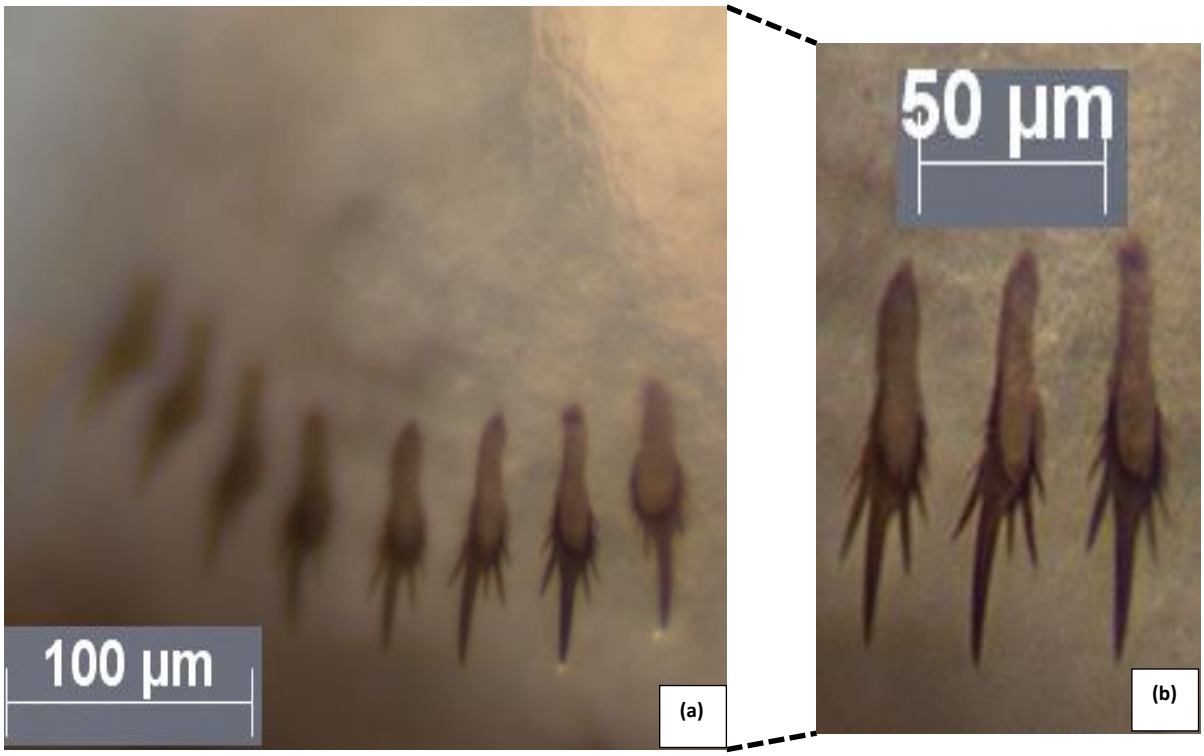

Figure 2.6: *Aedes aegypti* comb scales (a). Enhanced view of *Ae. aegypti* comb scales showing multiple subapical spines and one large apical spine (b).

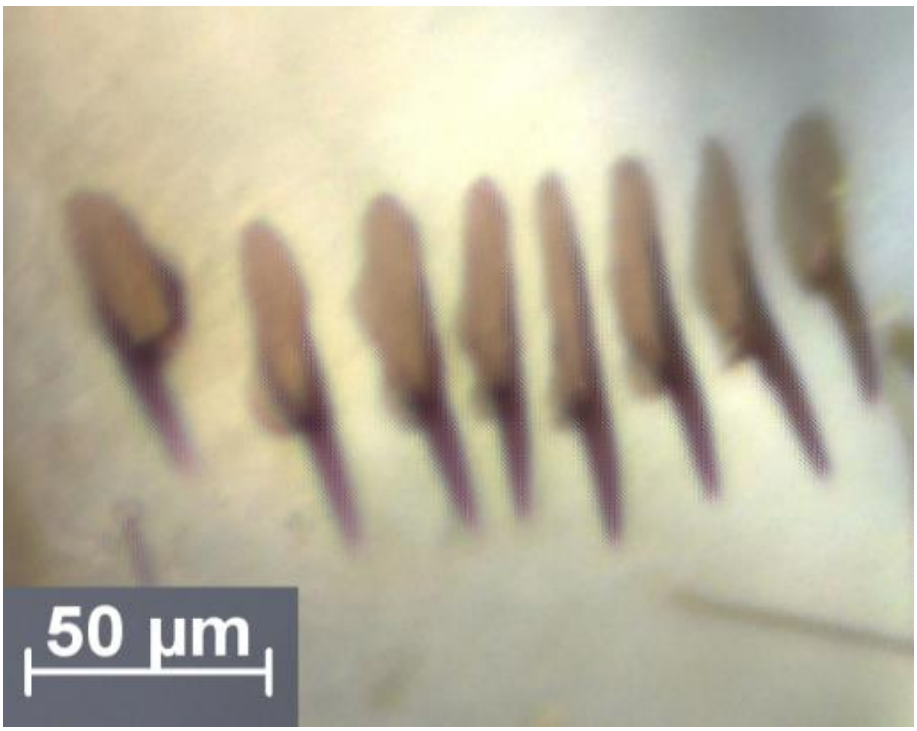

Figure 2.7: *Aedes albopictus*: comb scales without subapical spines.

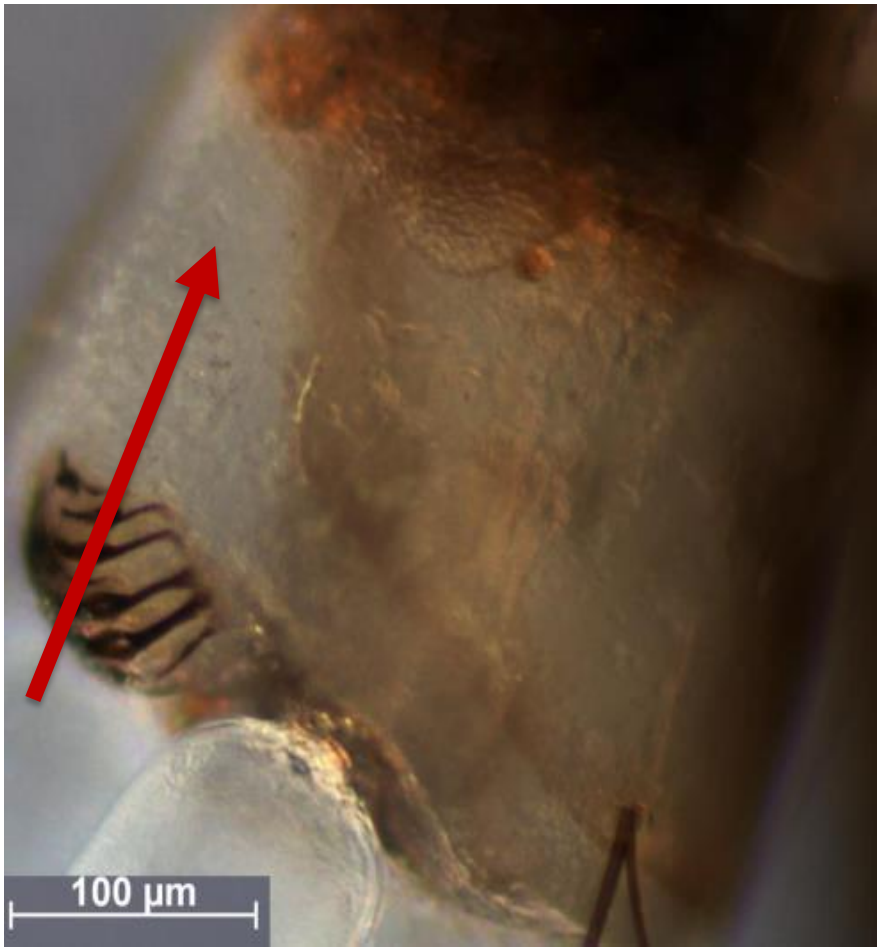

Figure 2.8: *Aedes albopictus*: Saddle of anal segment incomplete with a colourless, smooth patch ventrally interrupted between its ends (as indicated by red arrow).

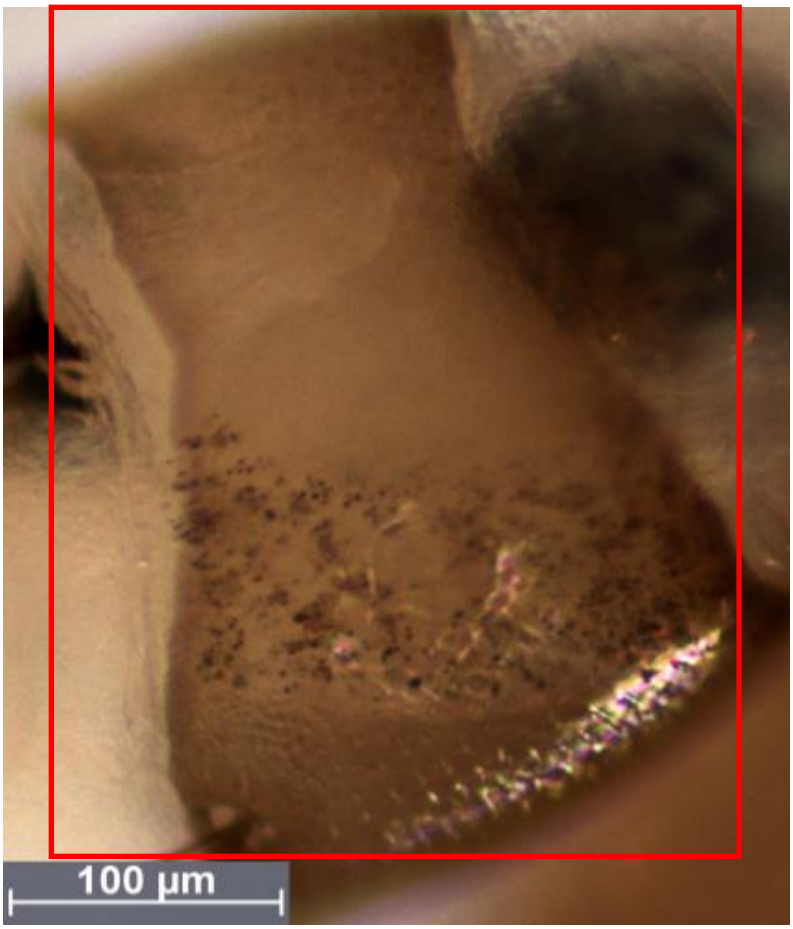

Figure 2.9: *Aedes tongae*: saddle of the anal segment complete (as indicated by box).

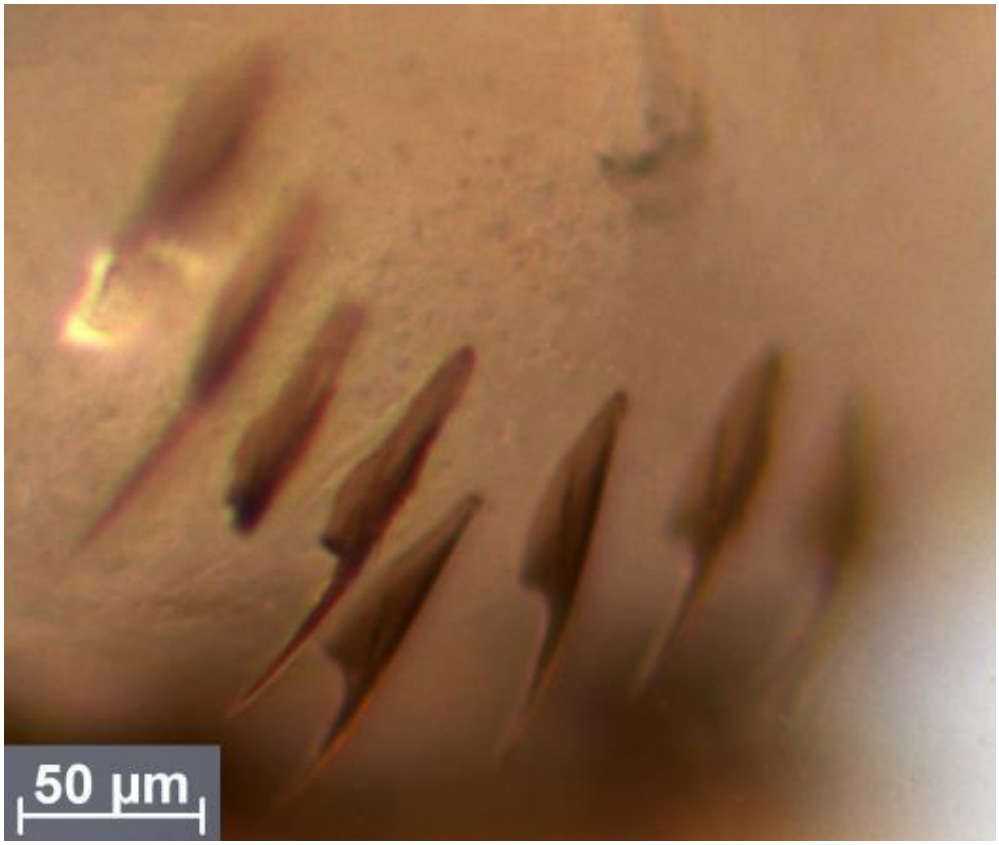

Figure 2.10: *Aedes vexans nocturnus* comb scales.

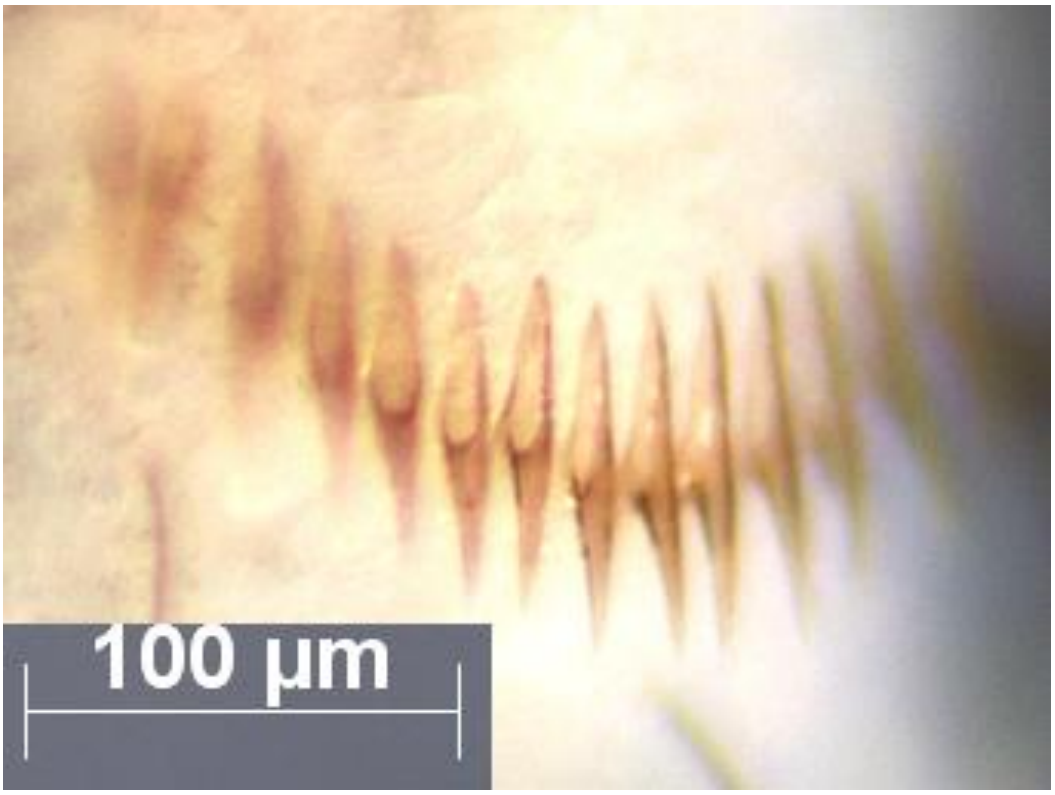

Figure 2.11: *Aedes horrescens*: sharp pointed comb scales in a slightly curved row.

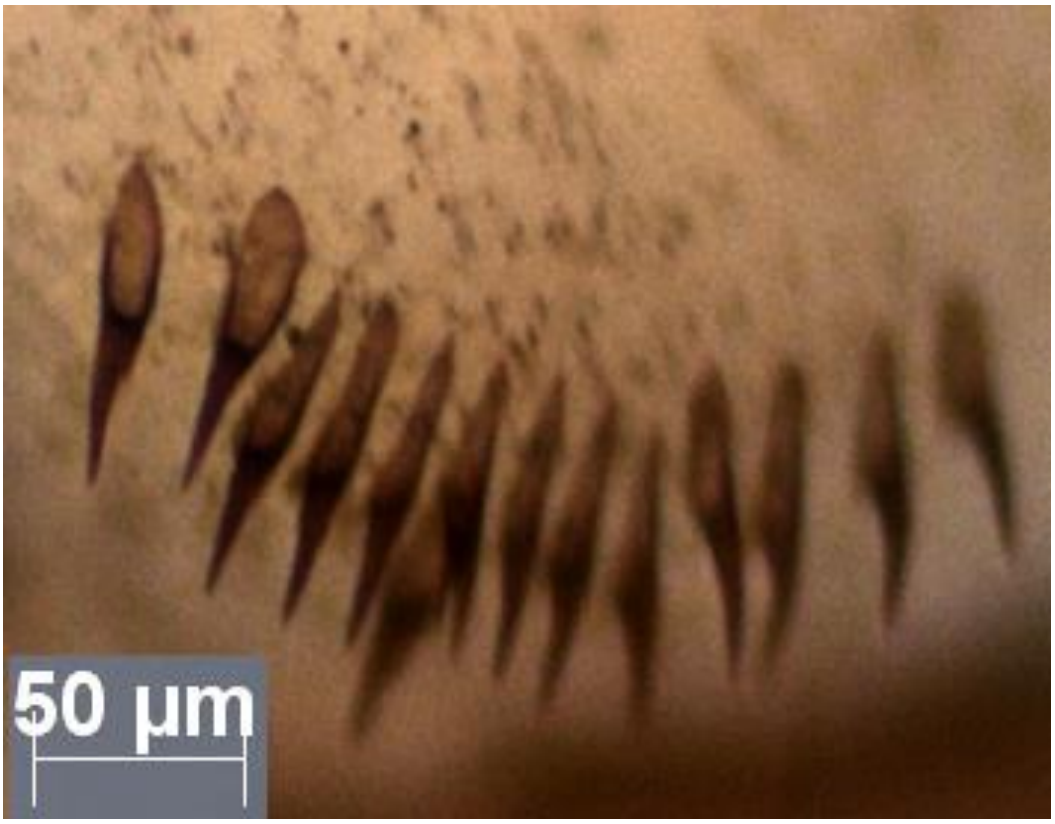

Figure 2.12: *Aedes tongae*: 8-14 sharp-pointed comb scales.

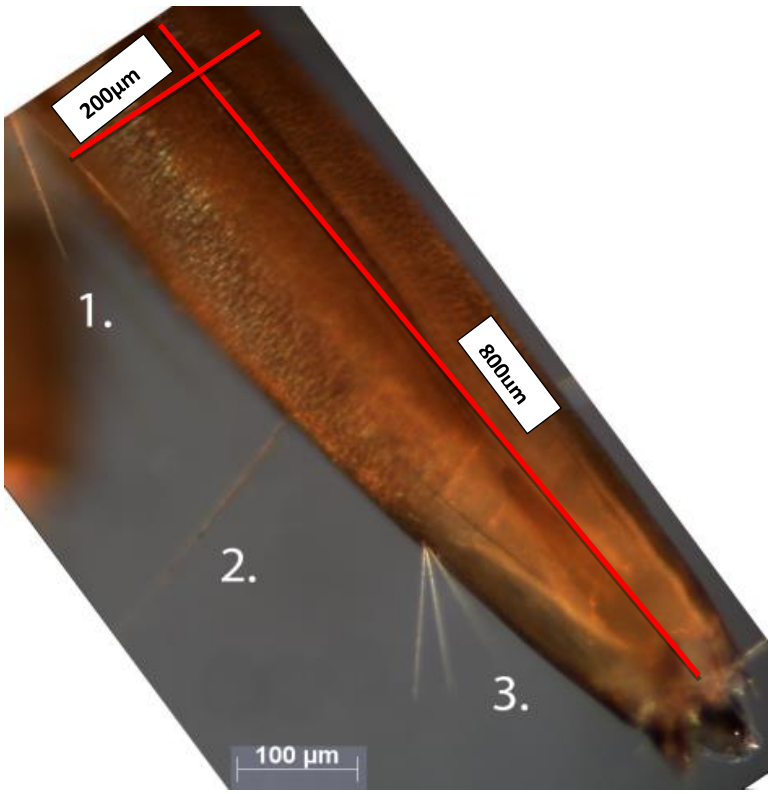

Figure 2.13: *Culex albinervis*: 3 pairs of subventral tufts of long hair. Siphon length about 9 times its mid-width (only part of the siphon is shown in this figure).

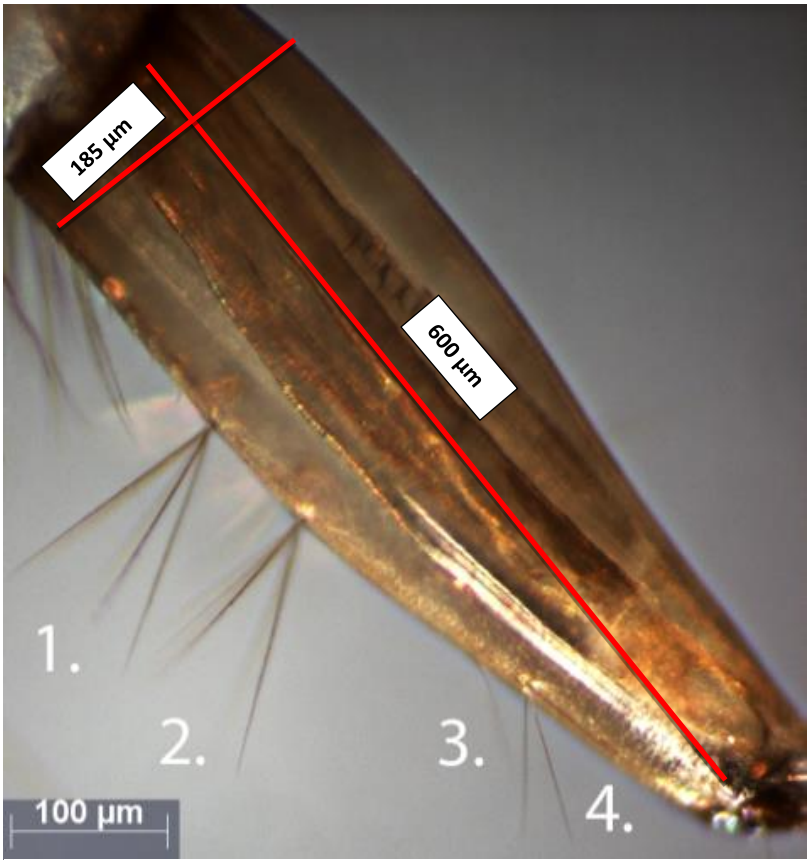

Figure 2.14: *Culex quinquefasciatus*: 4 pairs of subventral tufts of long hairs. Siphon length about 3.5 times its mid-width.

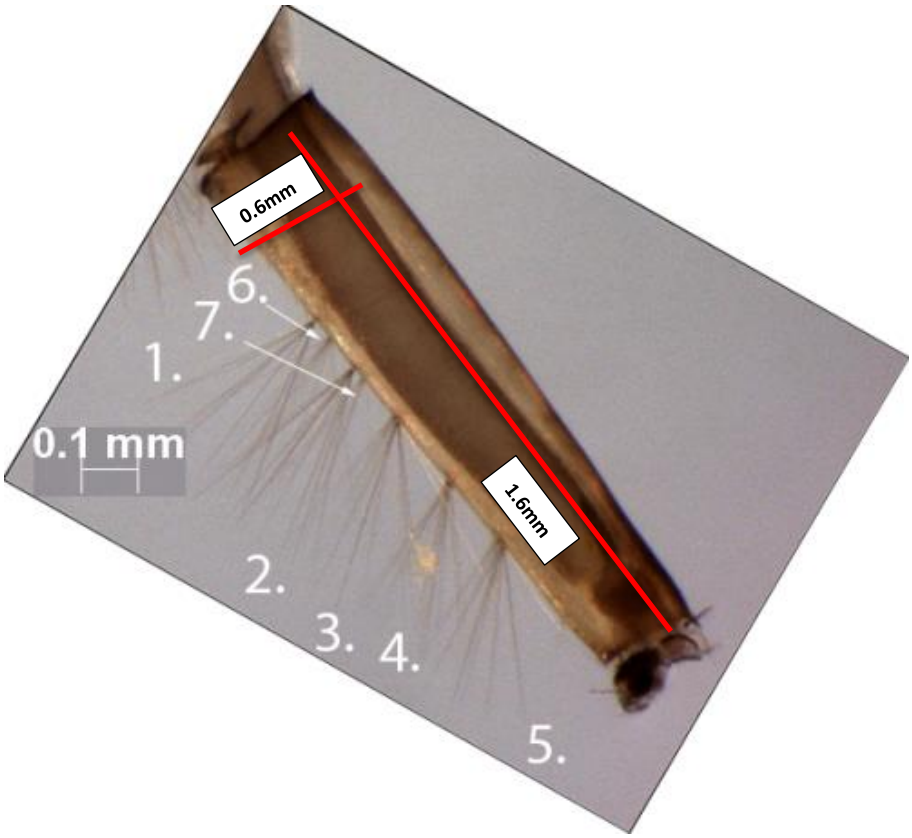

Figure 2.15: *Culex annulirostris*: 5-7 pairs of subventral tufts of long hair. Siphon length about 3.5 times its mid-width.

Figure 2.16: *Culex sitiens*: 5-7 subventral tufts of hair; Narrow siphon length.

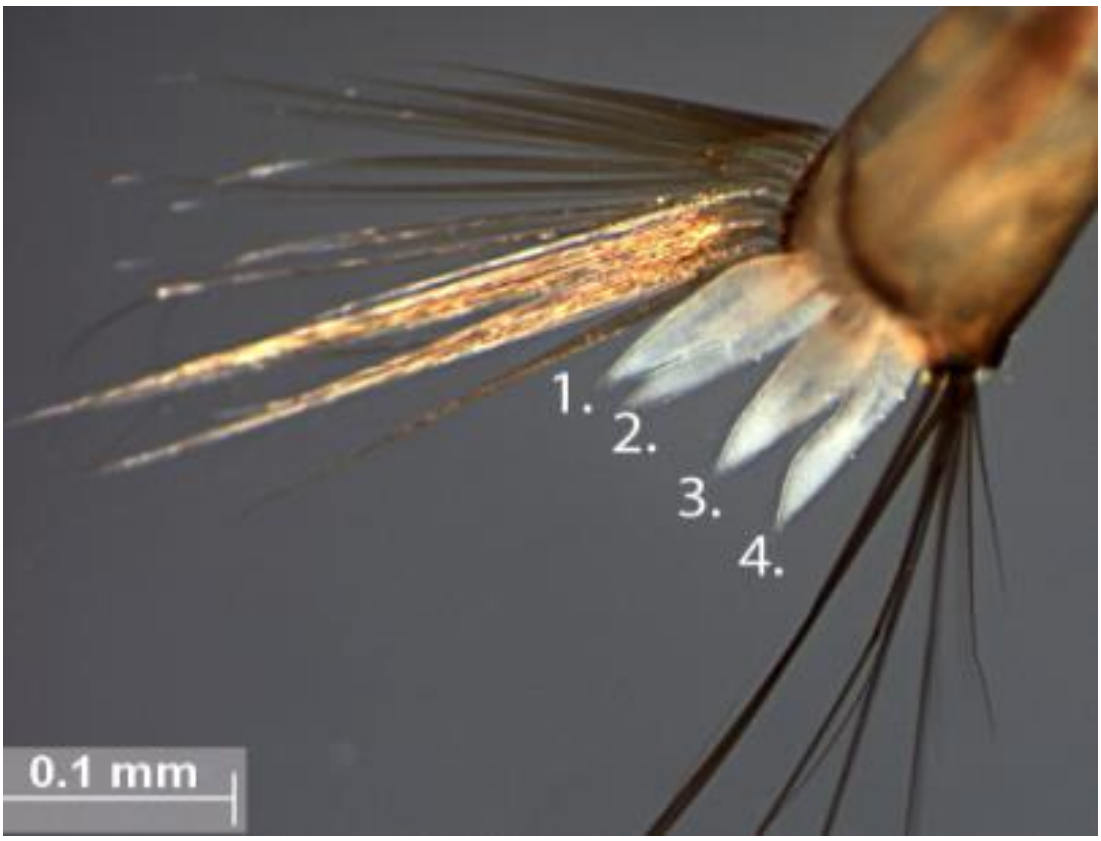

Figure 2.17: *Culex sitiens*: Anal gills (1-4) short and tapered.

Discussion

Although six published mosquito surveys have been undertaken in Tonga (Edwards et al. 1926; Laird 1956; Belkin 1962; Ramalingam 1976; Harding et al. 2007 and Guillaumot et al. 2012), *Ae. albopictus* was only recognised in the last of these. Its apparent absence in earlier surveys may have been an artefact due to technological limitations of the microscopes used for larval identification in those earlier times. It is likely that *Ae. albopictus* has been present in Tonga for many years and that Harding et al. (2007) may have misidentified it as either *Ae. aegypti* or *Ae. tongae* due to the lower magnification microscope available to them (J. Harding, personal communication, 20 March 2014). *Ae. aegypti* and *Ae. tongae* can be distinguished at larval stages owing to clear taxonomic differences such as comb scales with subapical spines for *Ae. aegypti* but comb scales without subapical spines in *Ae. albopictus* and *Ae. tongae*. The saddle of *Ae. tongae* is complete on the anal segment, but ventrally interrupted with a smooth surface for *Ae. albopictus*. The origins and arrival of *Ae. albopictus* into the Tongatapu Island Group will be discussed in more detail in the following Chapter.

Edwards (1935) described *Ae. horrescens* as having promient stellate setae in the larval form, which makes them appear hairy. In the past this distincitive feature has been used to identify this species, but in our specimens, the arrangement of the comb scales in a slightly curved row was a far more prominent and diagnostic feature.

Lastly, it needs to be recognised that technological limitations can reduce the ability to make positive identifications of mosquito larvae because of the very small sizes of many of the characters used for species discrimination. In particular, the shape of comb scales can be a critical feature. With respect to the Tongan fauna this is especially the case in distinguishing among *Ae. aegypti, Ae. albopictus* and *Ae. tongae*. Researchers should also be aware that additional exotic mosquito species, not included in this guide may also become established in the future.

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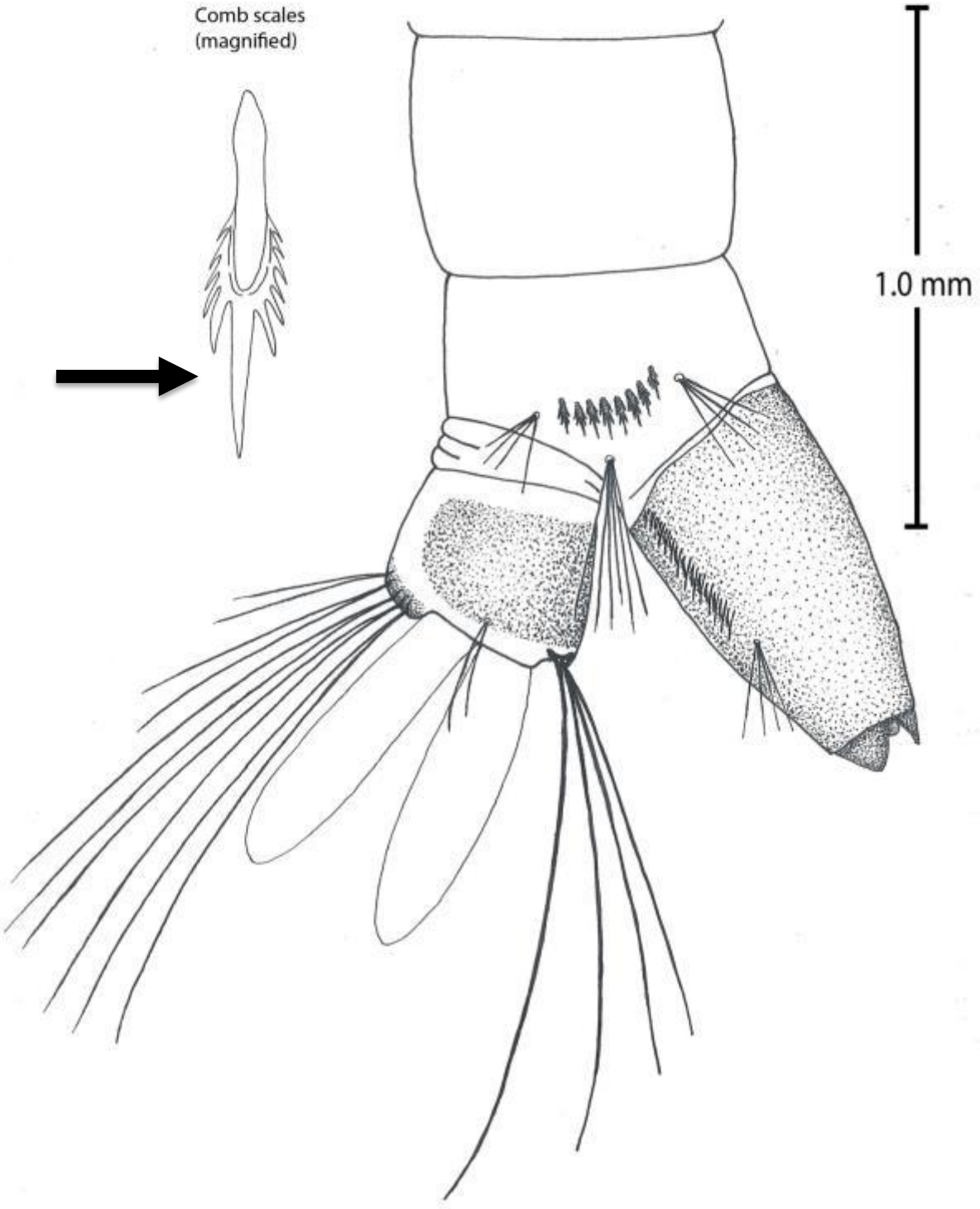
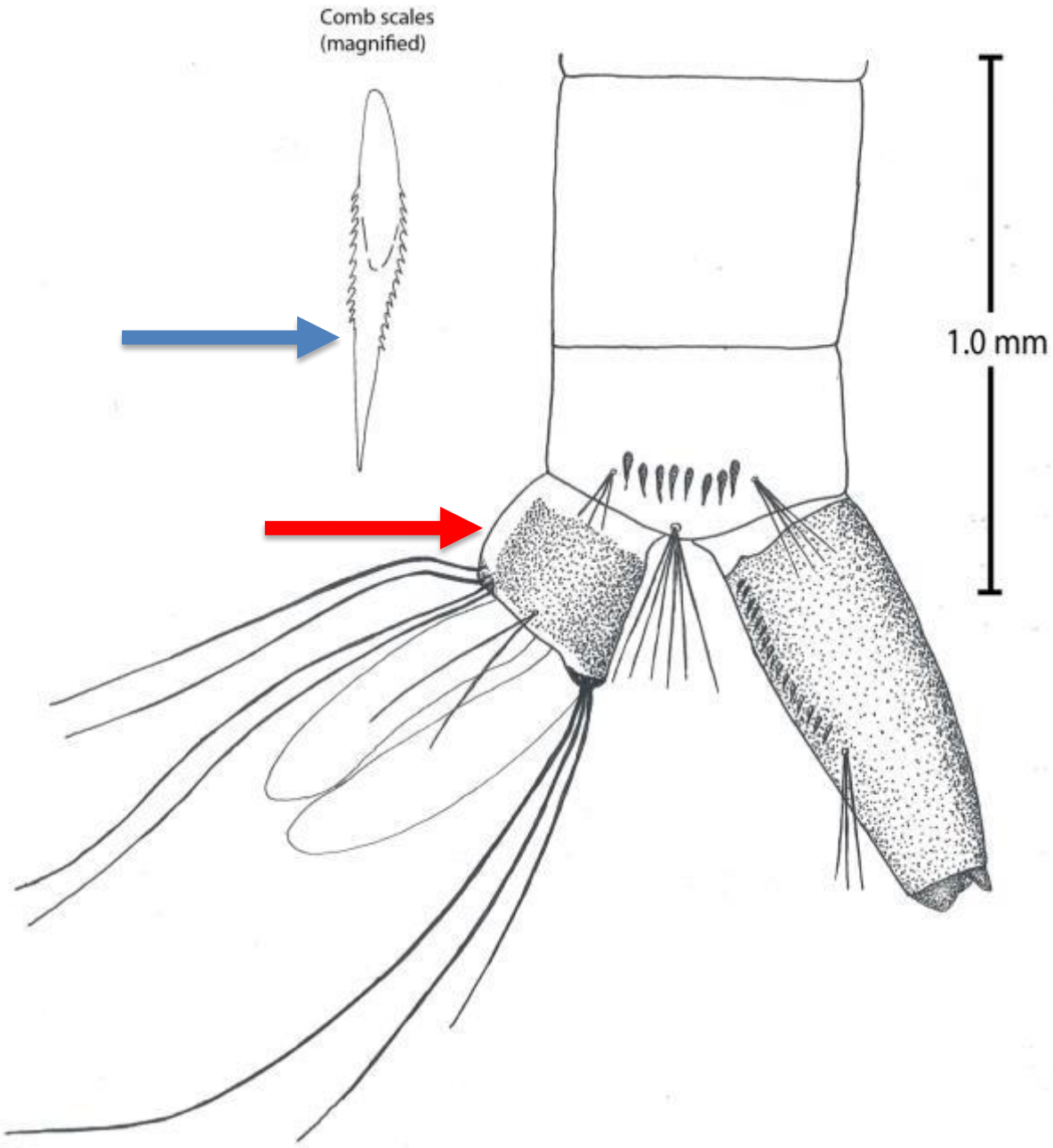
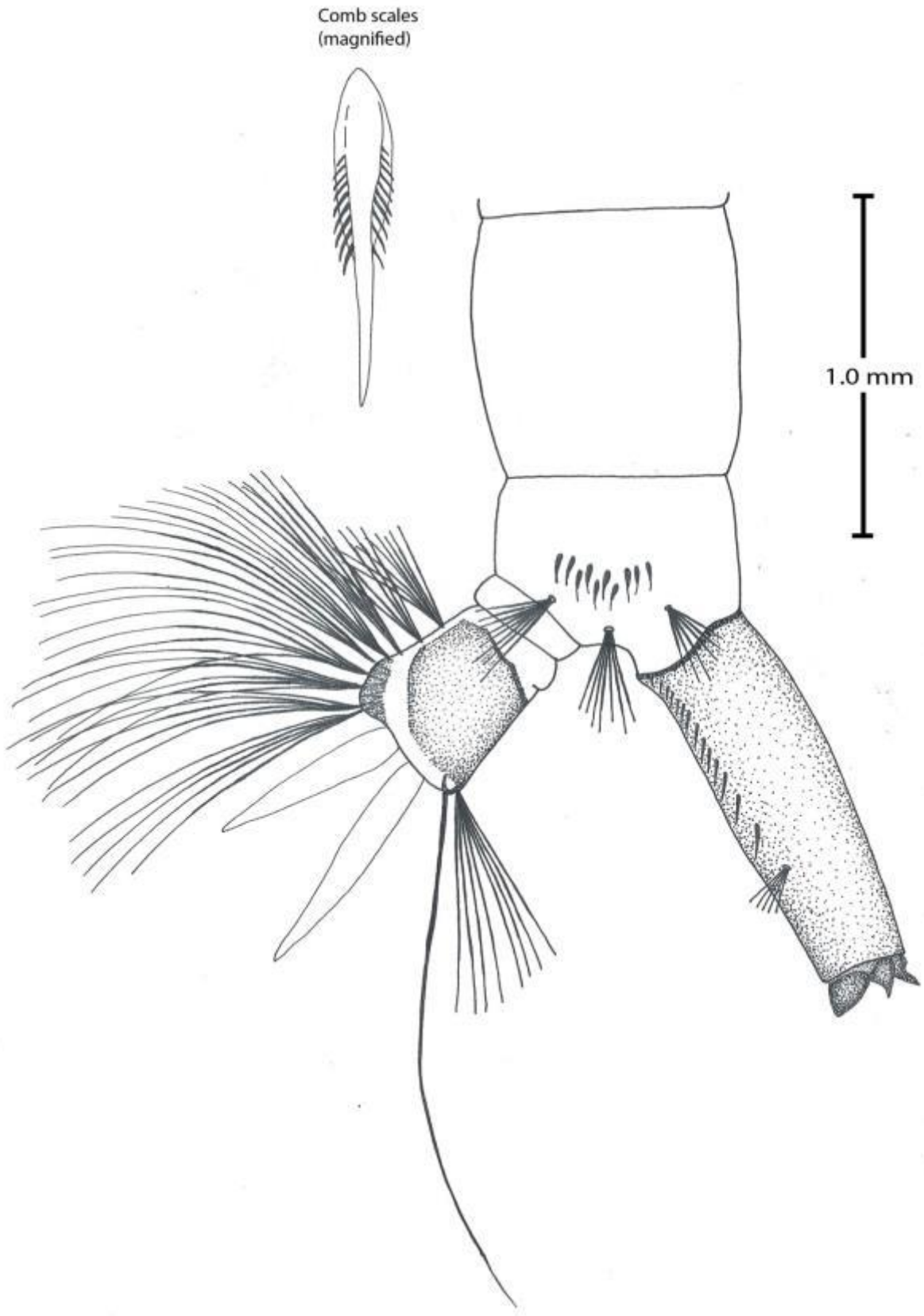
Appendices: Handrawn by Mark Galatowitsch based on drawings by Belkin (1962) and Rueda (2004).



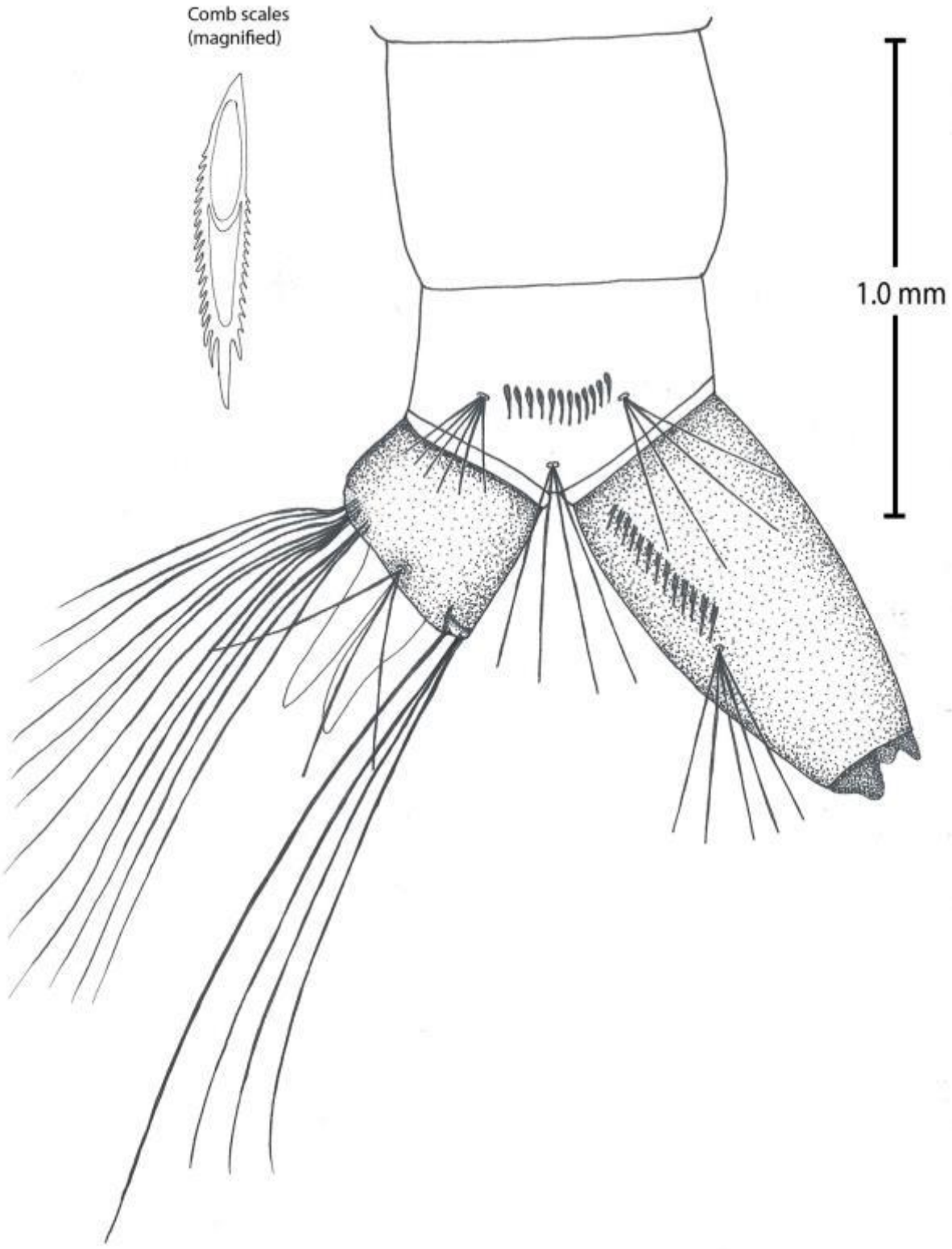
Appendix 2.1: Distal view of *Ae. aegypti* fourth instar larvae showing a row of 5-10 comb scales with about 5 subapical spines, one being much longer and thicker than the others.



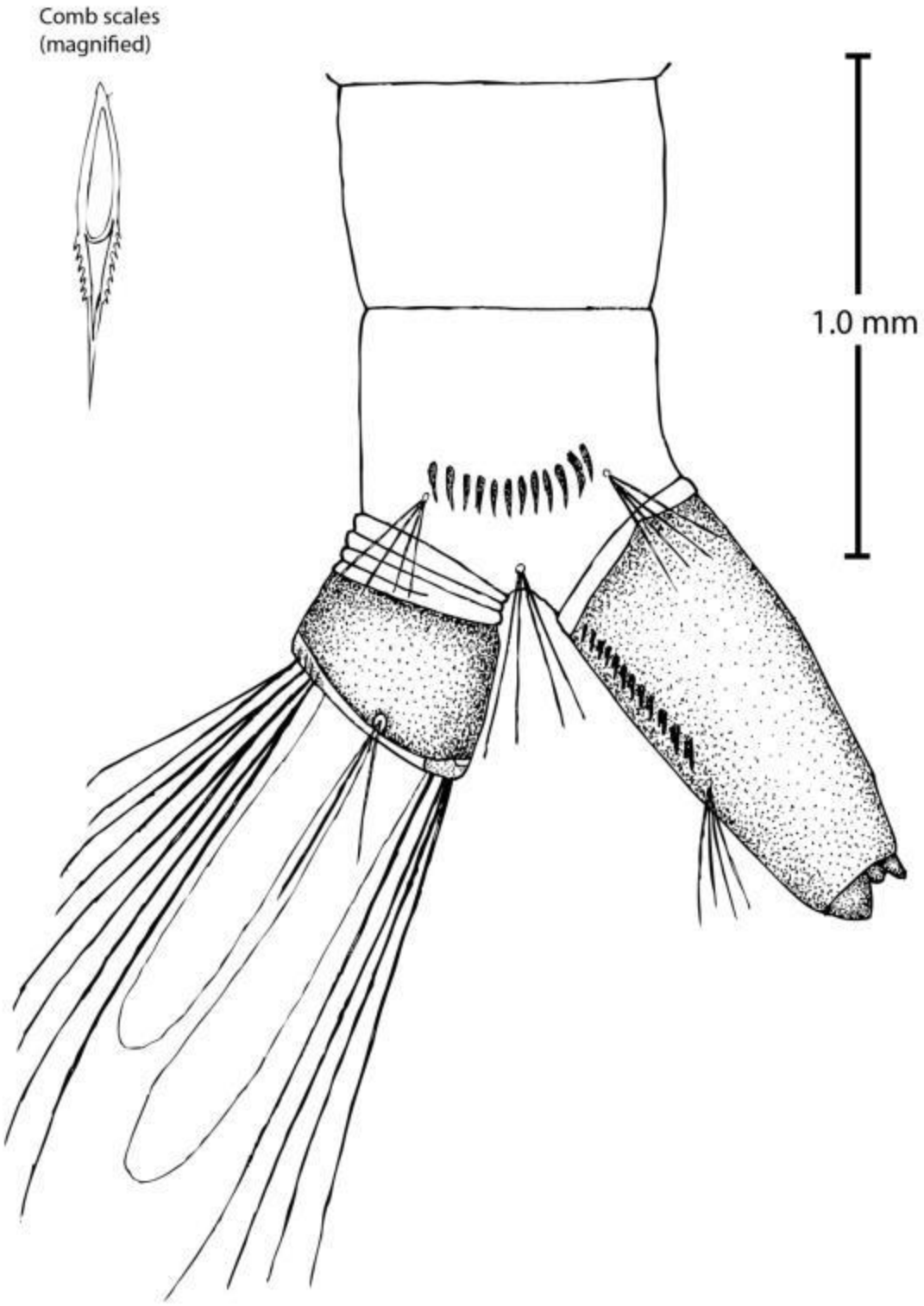
Appendix 2.2: Distal view of *Ae. albopictus* fourth instar larvae showing the saddle of anal segment interrupted ventrally by a smooth surface (red arrow) and about 8 comb scales in a straight line, the scales narrowing abruptly at about mid-length (blue arrow).



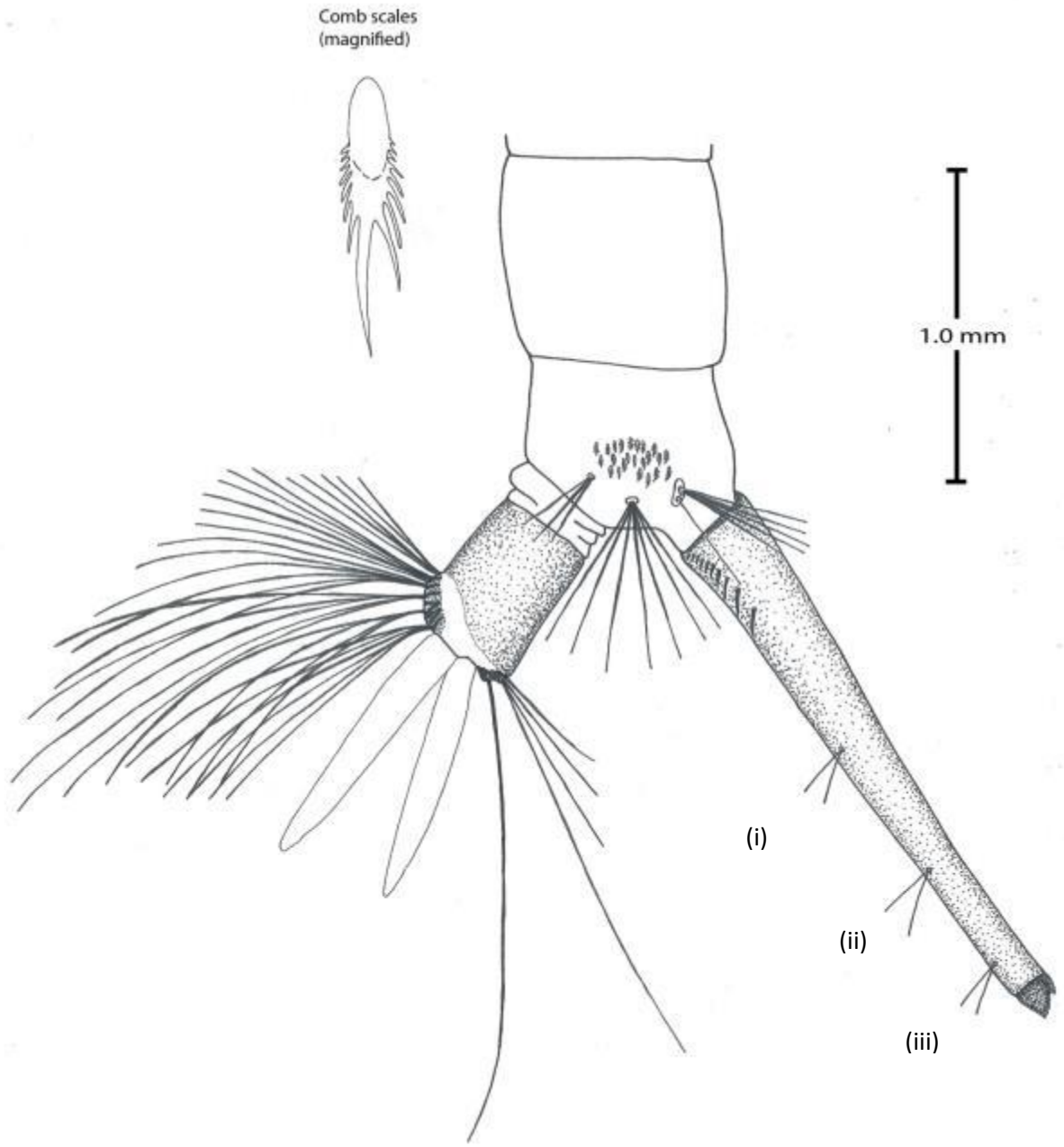
Appendix 2.3: Distal view of *Ae. vexans nocturnus* fourth instar larvae showing 8-10 comb scales in a jagged, roughly V-shaped line. Anal gills narrow and pointed.



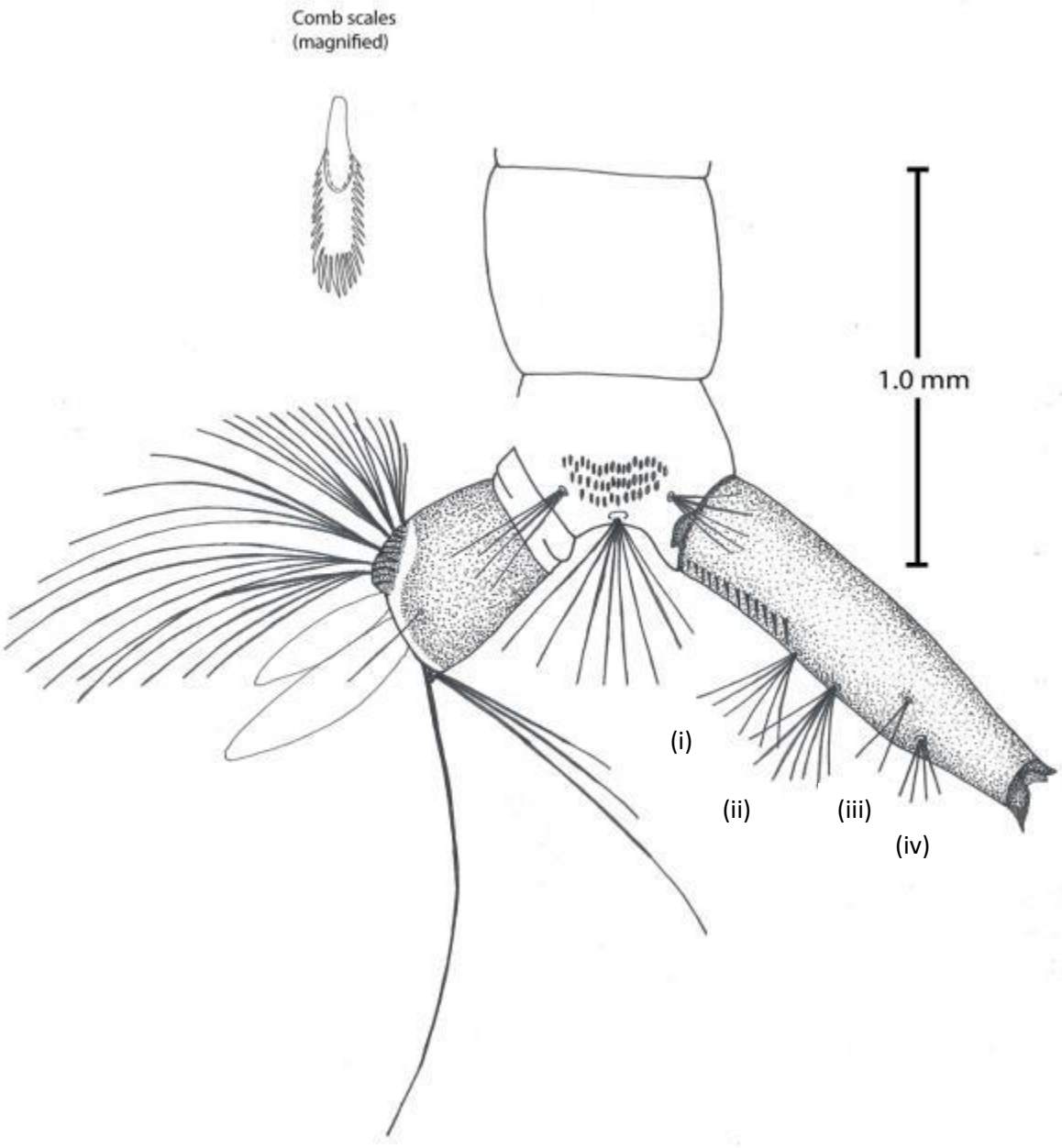
Appendix 2.4: Distal view of *Ae. horrescens* fourth instar larvae showing the line of 8-12 comb scales and anal gills not much longer than anal segment, their tips rounded.



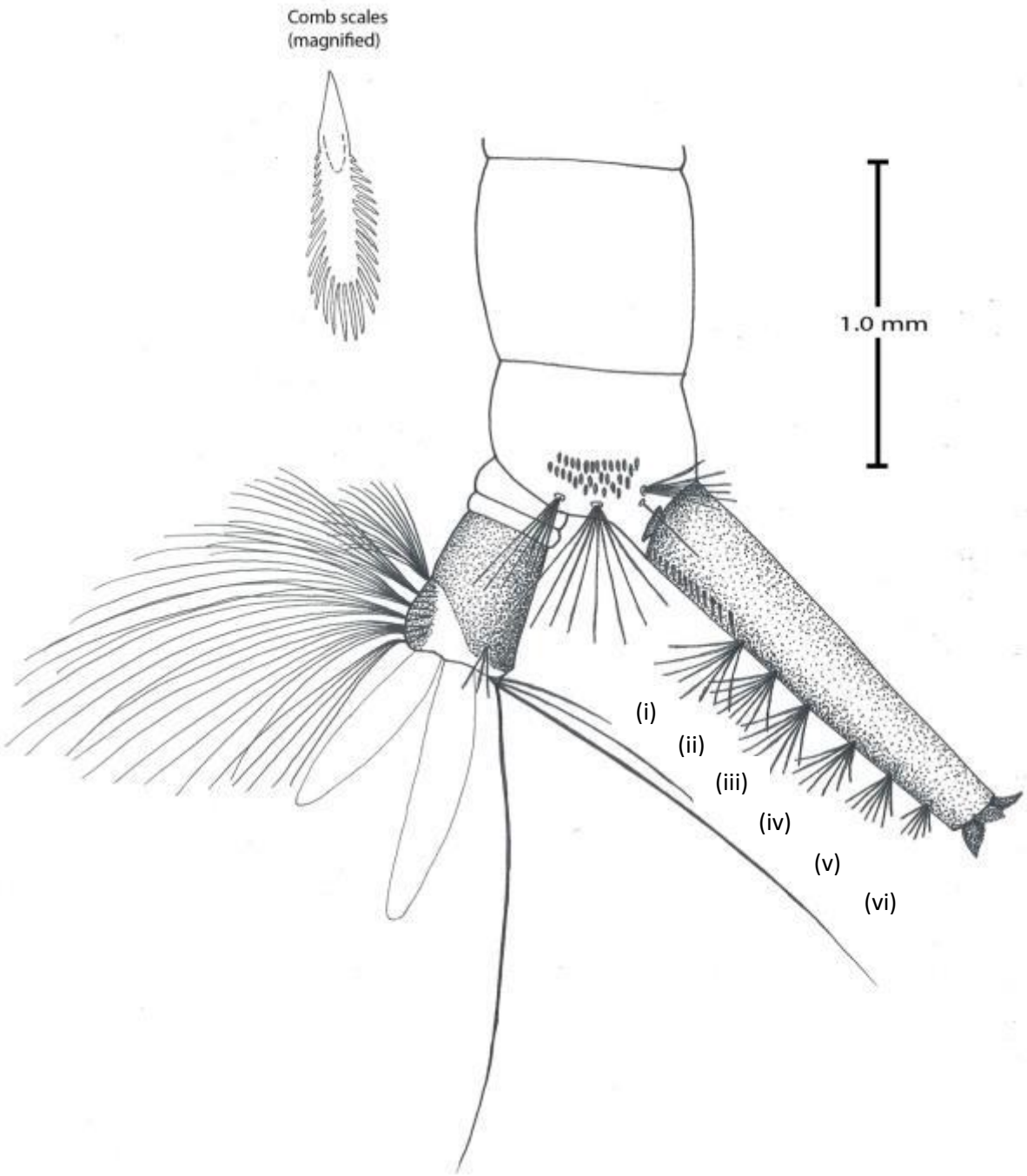
Appendix 2.5: Distal view of *Ae. tongae* fourth instar larvae showing 8-12 narrow, sharp pointed comb scales and anal gills 2-3 times longer than anal segment, their tips rounded.



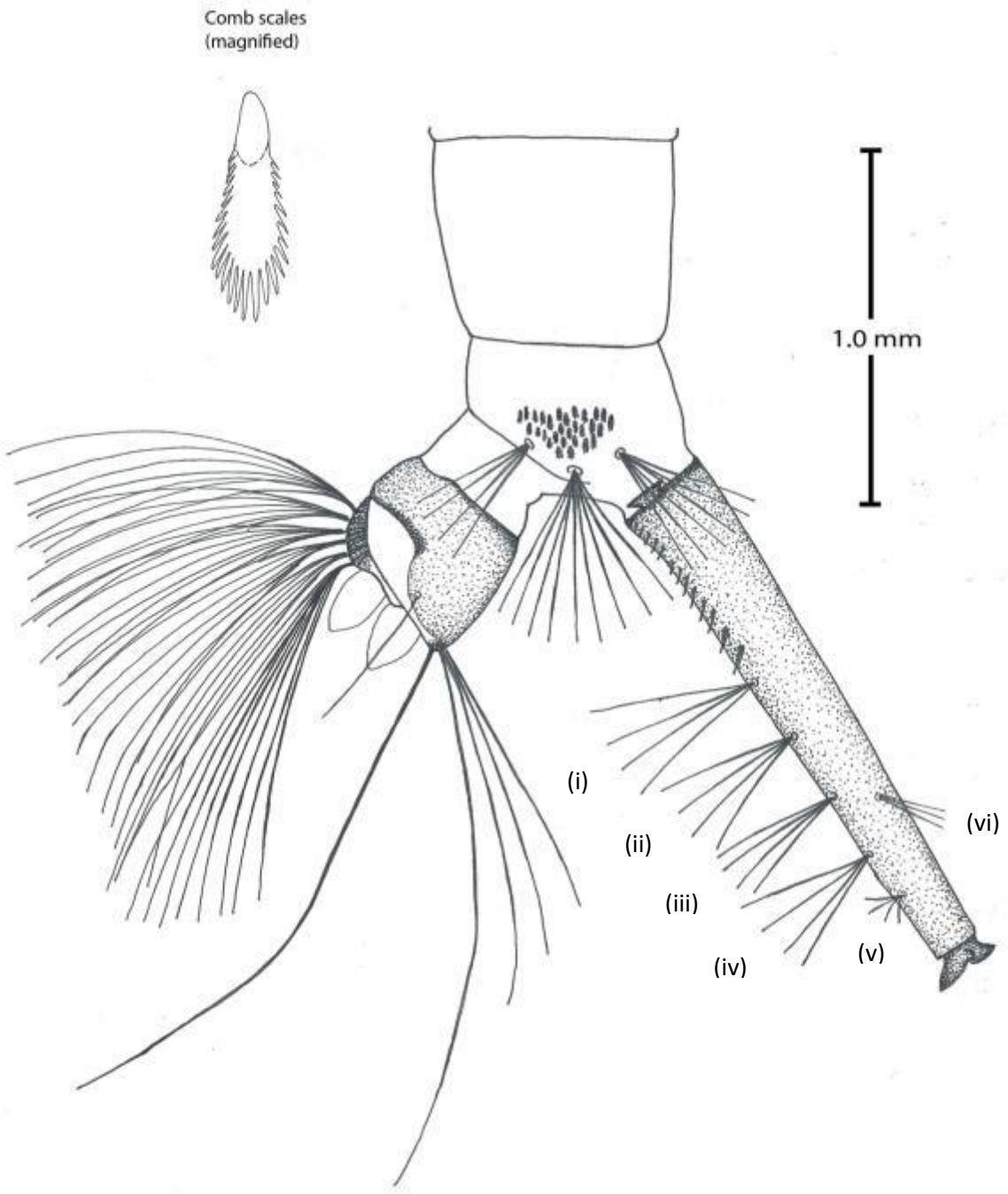
Appendix 2.6: Distal view of *Cx. albinervis* fourth instar larvae showing 3 pairs of subventral hairs (i-iii) and narrow length (about 9 times mid-width) of siphon.



Appendix 2.7: Distal view of *Cx. quinquefasciatus* fourth instar larvae showing 4 pairs of subventral hairs (i-iv) and siphon length about 3.5 times its mid-width.



Appendix 2.8: Distal view of *Cx. annulirostris* fourth instar larvae showing 5-7 tufts of long subventral hairs (i-vi) and siphon length 5-7 times its mid-width.



Appendix 2.9: Distal view of *Cx. sitiens* fourth instar larvae showing the 5-6 tufts of long subventral hairs (i-vi) and narrow siphon length 7-8 times its mid-width.

Chapter Three: The distribution of mosquito larvae in 2006 and 2013 in the Tongatapu Island Group

Abstract

Eighty-four sites across the four islands of Tongatapu, Pangaimotu, ‘Oneata, and ‘Eua in the Tongatapu Island Group were visited for a mosquito survey in November–December 2013. The purpose of this survey was to determine the current number of mosquito species present in the group since the last comprehensive survey in 2006. Nine mosquito species were collected: *Aedes aegypti* Linnaeus*, Ae. albopictus* Skuse, *Ae. tongae* Edwards, *Ae. horrescens* Edwards, *Ae. vexans nocturnus* Theobold*, Culex annulirostris* Skuse, *Cx. albinervis* Edwards, *Cx. quinquefasciatus* Say and *Cx. sitiens* Wiedemann, compared to eight mosquito species collected in 2006 (*Aedes aegypti*, *Ae. tongae*, *Ae. horrescens*, *Ae. vexans nocturnus*, *Culex annulirostris*, *Cx. albinervis*, *Cx. quinquefasciatus*, *Cx. sitiens*). This is only the second time that *Ae. albopictus* has been collected in the Tongatapu Island Group. *Aedes aegypti*, *Ae. albopictus,* and *Cx. quinquefasciatus* were collected on Tongatapu, Pangaimotu and ‘Eua, and *Ae. albopictus,* and *Cx. quinquefasciatus* on Tongatapu, ‘Oneata, and ‘Eua Islands. *Aedes tongae* and *Cx. annulirostris* were collected on Tongatapu and ‘Eua, and Tongatapu and ‘Oneata, respectively, and *Cx. albinervis, Cx. sitiens*, and *Ae. vexans nocturnus* were found on Tongatapu Island only. *Aedes horrescens* was taken from ‘Oneata Island only. *Aedes aegypti*, *Ae. albopictus, Ae. tongae*, and *Cx. quinquefasciatus* were widespread on Tongatapu and ‘Eua Islands, and have significantly increased their distributions on Tongatapu Island since surveyed in 2006. Conversely, the distribution and occurrence of *Cx. albinervis* and *Ae. vexans nocturnus* had decreased since 2006. *Culex sitiens* was rare in both 2006 and 2013 being limited to Tongatapu Island only. Similarly, *Ae. horrescens* was rare; it was found only on ‘Oneata Island in 2013 and on Tongatapu in 2006. The results of the 2013 survey indicate that the distribution of mosquitoes, particularly *Ae. aegypti*, *Ae. albopictus, Ae. tongae*, and *Cx. quinquefasciatus* have expanded rapidly

throughout the Tongatapu Island Group since 2006. This expansion may be a consequence of more widespread habitat availability (e.g., used car tyres, fuel drums, containers) throughout the islands.

Introduction

The global expansion of international air, sea, and land transport networks has greatly enhanced the spread of mosquitoes around the world (Reiter 1998). Additionally, human-mediated transport of mosquito eggs in used car tyres has distributed mosquitoes to geographic locations (such as landmasses separated by a geographic barrier, and islands) which were previously inaccessible (Tatem et al. 2006). Morphological traits of mosquitoes in the tribe *Aedini*, such as *Aedes albopictus* Skuse have drought resistant eggs, which enable survival until water is deposited into its habitat (e.g., a car tyre) in which the eggs can hatch (Enserink 2008). Traits such as this increase this species ability to tolerate tough-drought like conditions. This may be particularly useful in tropical countries that experience distinctive 'wet' and 'dry' seasons.

Poor refuse disposal is a significant problem in Pacific Island countries. As well as causing environmental, health, and aesthetic problems (Barnett and Campbell 2010), refuse such as used car tyres, fuel drums, and containers can provide habitats for mosquito larvae. Not surprisingly, a high density of human refuse in urban areas in the Solomon Islands resulted in dengue fever outbreaks as a consequence of an increased number of mosquitoes in that area (Inter Press Service News Agency 2015). Human refuse has therefore been shown to be a driver of mosquito occurrence and distribution, along with factors such as land use, elevation, human population density, and temperature (Gibbs et al. 2006).

The Tongatapu Island Group has a significant mosquito problem, which causes both nuisance and medical issues (Stanley 1999). Nevertheless, surprisingly few studies have been conducted on mosquitoes in Tonga. The earliest published mosquito study in Tonga was the description of *Ae. tongae* by Edwards (1926). Thirty years later Laird (1956) noted the presence of four species on Tongatapu (*Ae. vexans nocturnus* Theobold*, Ae. oceanicus* Belkin, *Culex annulirostris* Skuse, and *Cx.*

quinquefasciatus Say), and in 1960 Iyengar recorded eight species from Nuku'alofa, Tongatapu and Vava'u. Belkin (1962), who travelled extensively throughout the South Pacific published the most comprehensive work to date for Tonga. Ten species were collected, including the sole endemic species *Ae. tongae*, two introduced species *Ae. aegypti* Linnaeus and *Cx. quinquefasciatus,* two indigenous species *Cx. annulirostris* and *Cx. sitiens* Wiedemann, and two species spread by humans *Ae. vexans nocturnus* and *Ae. oceanicus*. Subsequently, Ramalingam (1976) collected eight of these species and in 1980 Huang and Hitchcock collected *Ae. kesseli*, *Ae. cooki*, and reclassified *Ae. tabu* as a subspecies of *Ae. tonage*. More recently, Harding et al. (2007) collected the larvae of eight mosquito species across 42 sites in the Tongatapu and Vava'u Island groups and, Guillaumot et al. (2012) recorded *Ae. albopictus* from Nuku'alofa and Vaiola in Tongatapu. The 16 species collected in Tonga over time is reviewed in Table 3.1 and a detailed list of species recorded by Harding et al. (2007) and Guillaumot et al. (2012) are reviewed in Table 3.2.

Based on the information above, the number of mosquito species present in Tonga seems to be changing, new species seem to be invading and some species may be disappearing. However, determining accurate and robust species lists, and defining the distributions of individual species has been difficult due to infrequent surveys of limited scope. The aim of this chapter is to compare species distributions in the Tongatapu Island Group in the 2013 survey, with the results of the survey conducted by Harding et al. in 2006.

Table 3.1: Mosquito species collected in Tonga over time. The report of *Ae. albopictus* in 2012 was not part of a full mosquito survey. * indicates a reclassification of *Ae. tabu* to subspecies level for *Ae. tongae* (Huang and Hitchcock 1980).

Years	Species	Number of new species	Total number of species	Author
1926	*Ae. tongae*	1	1	Edwards
1956	*Ae. vexans nocturnus, Ae. oceanicus, Cx. annulirostris, Cx. quinquefasciatus*	4	5	Laird
1960	*Ae. tongae, Ae. vexans nocturnus, Ae. aegypti, Ae. samoanus, Cx. annulirostris, Cx. sitiens, Cx. albinervis, Cx. pipiens fatigans*	5	10	Iyengar
1962	*Ae. tongae, Ae. vexans nocturnus, Ae. oceanicus, Ae. aegypti, Cx. annulirostris, Cx. sitiens, Cx. quinquefasciatus*	1	11	Belkin
1976	*Ae. tongae, Ae. vexans nocturnus, Ae. oceanicus, Ae. aegypti, Ae. tabu, Cx. annulirostris, Cx. sitiens, Cx. quinquefasciatus*	1	12	Ramalingam
1980	*Ae. kesseli*, Ae. cooki*, Ae. tongae*, Ae. tabu** [1]	2	14	Huang & Hitchcock
2006	*Ae. tongae, Ae. vexans nocturnus, Ae. aegypti, Ae. horrescens, Cx. annulirostris, Cx. sitiens, Cx. albinervis, Cx. quinquefasciatus*	1	15	Harding et al.
2012	*Ae. albopictus*	1	16	Guillaumot et al.
2013	*Ae. tongae, Ae. vexans nocturnus, Ae. aegypti, Ae. horrescens, Ae. albopictus, Cx. annulirostris, Cx. sitiens, Cx. albinervis, Cx. quinquefasciatus*	0	16	This study

Table 3.2: Mosquito species currently present in Tonga with comments on their biology and the diseases associated with them. Note that the presence of *Aedes albopictus* was first reported in 2012. Table modified from Mackereth et al. (2007) and updated.

Species	**Location**	**Larval habitat**	**Adult behavior**	**Disease**	**Indigenous, endemic introduced?**
Aedes aegypti	Tongatapu, 'Eua, Ha'apai, Va'vau (Harding et al. (2007); This study).	Container breeder in natural (e.g., tree-holes, coconut shells, fern stumps) and artificial habitats (e.g., tyres, buckets, fuel gallons). Prefers large habitats (Lee et al. 1987).	Most commonly bites during late afternoon to sunset. Multiple feeding of female mosquito for single egg batch can occur (Lee et al. 1987). This species has been found to disperse up to 800m from the emergence site (Honório et al. 2003).	Lumpy skin disease in cattle, yellow fever, dengue fever, West Nile virus, chikungunya fever, Ross River virus (Belkin 1962; Ramalingam 1967).	Introduced by Europeans (Belkin 1962).
Aedes horrescens	Tongatapu (Harding et al. (2007); This study).	Found primarily in rural sites in Tonga (Harding et al. 2007).	No information available.	Dengue fever, filariasis (Belkin 1962; Ramalingam 1967).	Likely to be introduced (Belkin 1962).
Aedes vexans nocturnus	Tongatapu, Ha'apai (Harding et al. (2007); This study).	Found breeding in shallow, temporary pools, marshes, road side ditches and grassy pools (Johansen et al. 2004).	Observed as a common household species (Johansen et al. 2004). Bites ferociously at dusk and dawn (Nishida & Tenorio, 1993).	West Nile virus, Japanese encephalitis (Belkin 1962; Ramalingam 1967).	Introduced and spread by Tongans (Belkin 1962).
Aedes tongae	Tongatapu, 'Eua, Ha'apai, Va'vau (Harding et al. (2007); This study).	Found primarily in urban areas in Tonga (Harding et al. 2007).	No information available.	Filariasis (Belkin 1962; Ramalingam 1967).	Endemic (Belkin 1962).
Aedes albopictus	Tongatapu, 'Eua (Guillaumot et al. (2012); This study).	Breeds in natural (e.g., tree-holes, coconut shells, fern stumps) and artificial habitats (e.g., tyres, buckets, fuel gallons). Found in urban, suburban and forested environments. Larvae can tolerate brackish and polluted water. Eggs are desiccant resistant (Hawley 1998).	Female preference for ovipositing in urban and residential habitats. Females are aggressive biters during the day (Benedict et al. 2007). This species can disperse up to 180m (Lee et al. 1987) in its lifetime, and has been known to disperse up to 800m (Honório et al. 2003).	Dengue, yellow fever, chikungunya fever, encephalitis, Ross River virus. (Belkin 1962; Ramalingam 1967).	Introduced (Guillaumot et al. 2012).
Culex albinervis	Tongatapu (Harding et al. (2007); This study).	Breeds commonly in shallow, stagnant freshwater pools lying in a roadside ditch (Lee et al. 1989). Also found in streams and rock holes with filamentous green algae (Laird 1956).	No information available.	None known or suspected (Lee et al. 1989).	Likely to be introduced (Belkin 1962).
Culex annulirostris	Tongatapu (Harding et al. (2007); This study).	Freshwater habitats, riverine habitats, containers, brackish and polluted waters (Lee et al. 1989).	Female bites pre-dawn and in the evening (Lee et al. 1989).	Murray Valley encephalitis, Kunjin virus, Japanese encephalitis, Ross River Fever, Barmah Forest virus. (Belkin 1962; Ramalingam 1967).	Indigenous (Belkin 1962).
Culex quinquefasci-atus	Tongatapu, 'Eua, Ha'apai, Va'vau (Harding et al. (2007); This study).	Can breed in a wide variety of natural and artificial habitats, but prefers organic rich waters (Lee et al. 1987). Only collected in artificial habitats, primarily 44-gallon drums and concrete water tanks (Harding et al. 2007).	A common domestic pest in urban areas, particularly a nuisance indoors. Possible dispersal range of 0.8-5.6km (Fussell 1964).	Filariasis, Avian malaria, Murray encephalitis, Lymphatic disease (Belkin 1962; Ramalingam 1967)	Introduced and spread by Europeans (Belkin 1962).
Culex sitiens	Tongatapu, Va'vau (Harding et al. (2007); This study).	Typically breeds in coastal, brackish waters in pools formed by high tides and rainfall (Russell, 1993). Occasionally found in freshwater pools and in natural and artificial containers (Sirivanakarn 1976).	Nocturnal biters, but are also known to bite at sunset (Standfast 1967). Specimens have been caught up to 20km from their breeding sites (Darsie and Ward 1981).	Murray River encephalitis, Japanese encephalitis, Ross River virus (Belkin 1962; Ramalingam 1967).	Indigenous (Belkin 1962).

Methods

Study area and design

Four Islands were surveyed within the Tongatapu Island Group on a single occasion between November and December, 2013. The islands surveyed were Tongatapu, 'Eua, Pangaimotu, and 'Oneata (Figure 3.1).

Tongatapu Island

Tongatapu is the largest island (261 km^2) in the Kingdom and contains the only international airport in Tonga. Tongatapu is of volcanic and raised coral origin, resulting in low-lying topography with little hilly terrain. Tongatapu is the most populous island with 75,416 inhabitants (Tonga Department of Statistics 2011). The urban areas of Tongatapu consist of the capital city (Nuku'alofa) and approximately 45 smaller villages. Villages consist of 50–100 buildings, and are almost completely residential, with a few small businesses (small shopping centres, dairies). The rural areas consist of 5–10 households and are almost completely dominated by coconut trees and fields of cultivated crops such as coconut, taro, and sweet potato.

'Eua Island

'Eua Island (87 km^2) has the highest point in the Tongatapu Island Group (312 m above sea level) and is largely composed of limestone, but with a core of volcanic rock forming exposed outcrops along the Eastern ridges (Hoffmeister et al. 1932; Bryan et al. 1972). The population of 'Eua is 5,016 people (Tonga Department of Statistics 2011). 'Eua is located 42 km from Tongatapu and is accessible via boat or plane.

Pangaimotu Island

Pangaimotu is a small island (1.60 km^2) located 2–3 km from Nuku'alofa (Figure 3.1). The island has a population of less than 100 inhabitants but is heavily populated by visiting tourists.

'Oneata Island

'Oneata is a small (1.01 km^2) uninhabited island 3–4 km from Nuku'alofa (Figure 3.1).

Figure 3.1: The four islands surveyed in the Tongatapu Island Group; (a) Tongatapu Island, (b) 'Eua Island, (c) Pangaimotu Island, (d) 'Oneata Island. Image (e) Pangaimotu Island (i) and 'Oneata Island (ii) are 2–3 km and 3–4 km from Tongatapu Island. Image (f) 'Eua island (iii) is 42 km from Tongatapu Island (iv). Different scales are indicated. Images modified from Google Earth.

Sampling methods

A total of 68 sites were sampled on Tongatapu Island, 12 on 'Eua Island, three on Oneata Island, and one on Pangaimotu Island (Figures 3.2 and 3.3). Sites consisted of both artificial (e.g., used car tyres, fuel drums, containers) and natural (e.g., pools, ponds, tree holes) habitats. Within each sampling area, potential larval habitats were searched for in a 300 m circumference. A site is defined as a

location in which sampling occurred from a single habitat in any given village. No more than two sites were collected from within each 300 m circumference. If no habitats were found, local inhabitants were asked about possible larval habitats outside of it. On ‘Eua Island, larval habitats were searched for around the wharf and within the main village centres throughout the island. As Pangaimotu and ‘Oneata islands are small, larval habitats were looked for by walking the length of the island. At each site several features were recorded including habitat wetted length (cm), width (cm), and depth (cm) with a tape measure. Dissolved oxygen and temperature were measured with a YSI 550a water quality meter, and specific conductivity and pH with an Oakton pH/CON 10 meter. Mosquito larvae were preserved in the field in 50 ml vials of 70% ethanol and returned to the University of Canterbury laboratory for identification. As larval habitats could not always be sampled completely, only presence/absence data is reported here.

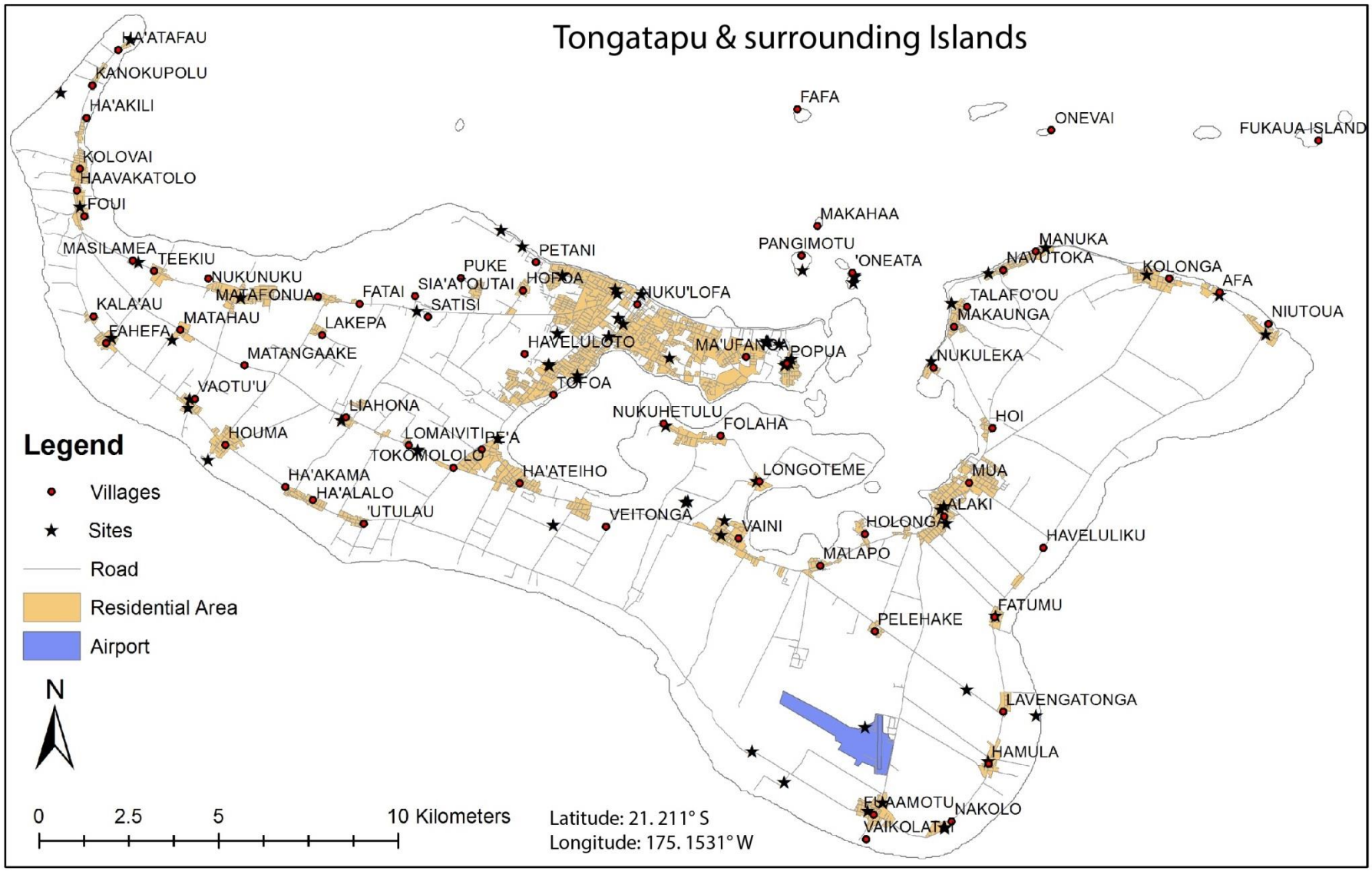


Figure 3.2: Tongatapu, Pangaimotu and 'Oneata Islands showing residential areas, villages, roads, airport, and sample sites (n=72) visited in November–December 2013. Only 60 points are shown as some sites were adjacent to each other.

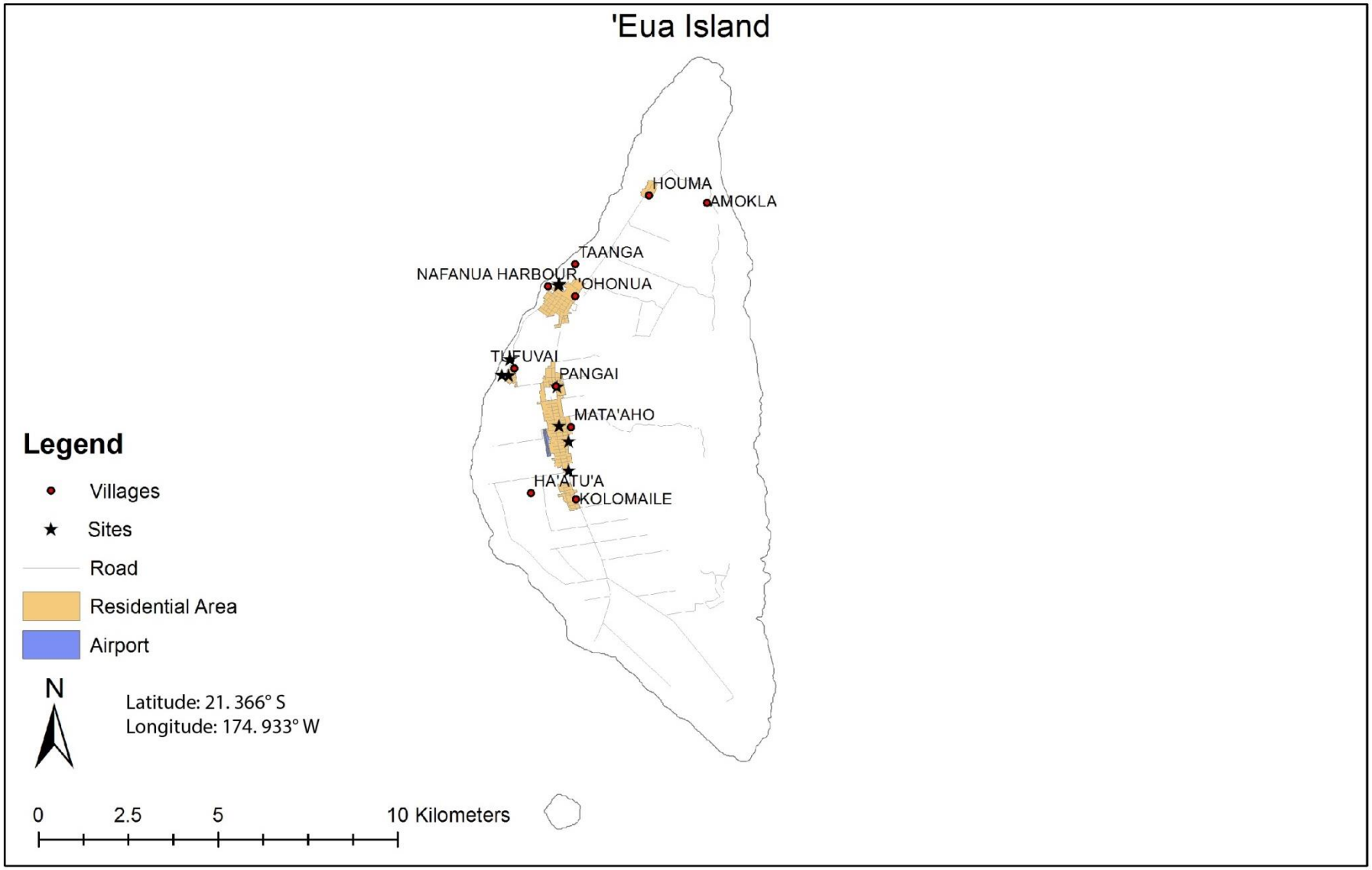


Figure 3.3: 'Eua Island showing residential areas, villages, roads, airport and the 12 sites visited in November–December 2013. Only eight points are shown as some sites were adjacent to each other.

Mosquito larvae and habitat sampling

Mosquito larvae were collected with pipettes, turkey basters and dippers depending on the water depth and accessibility of the habitat (Table 3.3). All larvae were preserved in the field in 50 ml vials of 70% ethanol. Vials were sealed with Whatman® Laboratory Sealing Film and returned to the University of Canterbury, New Zealand for laboratory identification.

Table 3.3: Sampling method used in different habitats within the Tongatapu Island Group.

Sampling method	Water depth	Habitat type
Mosquito dipper (350 ml)	> 200 ml	44 gallon drums, water tanks, buckets, ponds.
Turkey baster (100 ml)	< 200 ml	Car tyres, drains, ice cream containers.
Pipette (10 ml)	< 10 ml	Car tyres, wheel ruts.

Data analysis

Mosquito larvae were identified to species level in the laboratory and recorded in Microsoft Excel with accompanying GPS coordinates for each site. Data were then imported into ESRI ArcMap 10.2. Individual GIS layers were created for each species and matched with the site and GPS coordinates where they were found. The coordinate system used was WGS 1984. The Land Information New Zealand (LINZ) site was used to convert x, y coordinates into decimal degrees for use in ArcMap before importation. Where GPS coordinates could not be obtained, features were manually digitised from field notes taken at the time of sampling. Land information and village names were acquired from the Tongan Ministry of Health.

Results

A total of eight mosquito species were collected in the Tongatapu Island Group in 2006 (Harding et al. 2007), compared with nine mosquito species collected in the same Island Group in 2013 (Figure 3.4). Eight species were collected on Tongatapu Island, four on ‘Oneata Island, four species on ‘Eua Island, and one on Pangaimotu Island (Table 3.4).

Aedes aegypti was the most common species to occur (32% of all occurrences), followed by *Ae. albopictus* (29%), and *Cx. quinquefasciatus* (19%) which occurred on three out of the four islands sampled (Table 3.4). *Aedes tongae* (9%) and *Cx. annulirostris* (3.8%) were less common and collected on two islands, and *Ae. vexans nocturnus* (2.6%), *Cx. sitiens* (1.9%), *Cx. albinervis* (0.6%), and *Ae. horrescens* (0.6%) were relatively rare occurring only on one island (Table 3.4).

The distribution and occurrence of *Ae. aegypti* on Tongatapu Island appeared to be much greater in 2013 than in 2006 (Figure 3.5). Similarly, the distribution and occurrence of *Cx. quinquefasciatus*, and *Ae. tongae* were greater on Tongatapu Island in 2013 (Figures 3.6 and 3.7). Furthermore, *Ae. albopictus* was widespread throughout Tongatapu Island, since initially being recorded in two locations in 2012, and absent in 2006 (Figure 3.8). Conversely, fewer *Cx. albinervis* and *Ae. vexans nocturnus* were found than in 2006 (Figures 3.9 and 3.10). *Culex sitiens* was rare in both surveys and limited to Tongatapu Island. *Aedes horrescens* was also rare and although found on Tongatapu Island in 2006 was only found on ‘Oneata Island in 2013. *Aedes aegypti*, *Ae. albopictus*, *Ae. tongae* and *Cx. quinquefasciatus* were collected for the first time in ‘Eua Island in 2013 (Figure 3.11).

Table 1: Number of occurrences of each mosquito species (total mosquito species occurrences =154) per island: Tongatapu (n=68 sites), 'Eua (n=12 sites), 'Oneata (n=3 sites), and Pangaimotu (n=1 site). Total number of mosquito occurrences per island is based on species counts of presence/absent at each site. Percentages are calculated from the number of occurrences of individual species divided by total number of mosquito occurrences per island. * indicates the first record of this mosquito species for this island.

	Tongatapu	'Eua	'Oneata	Pangaimotu
Aedes albopictus	29% (n=38)	33% (n=6)*	20% (n=1)*	
Aedes aegypti	33% (n=43)	33% (n=6)*		100% (n=1)
Aedes horrescens			20% (n=1)*	
Aedes vexans nocturnus	3% (n=4)			
Aedes tongae	9% (n=12)	11% (n=2)*		
Culex albinervis	0.7% (n=1)			
Culex annulirostris	4% (n=5)		20% (n=1)*	
Culex quinquefasciatus	18% (n=24)	22% (n=4)*	40% (n=2)*	
Culex sitiens	2% (n=3)			
Total number of mosquito occurrences per island	130	18	5	1

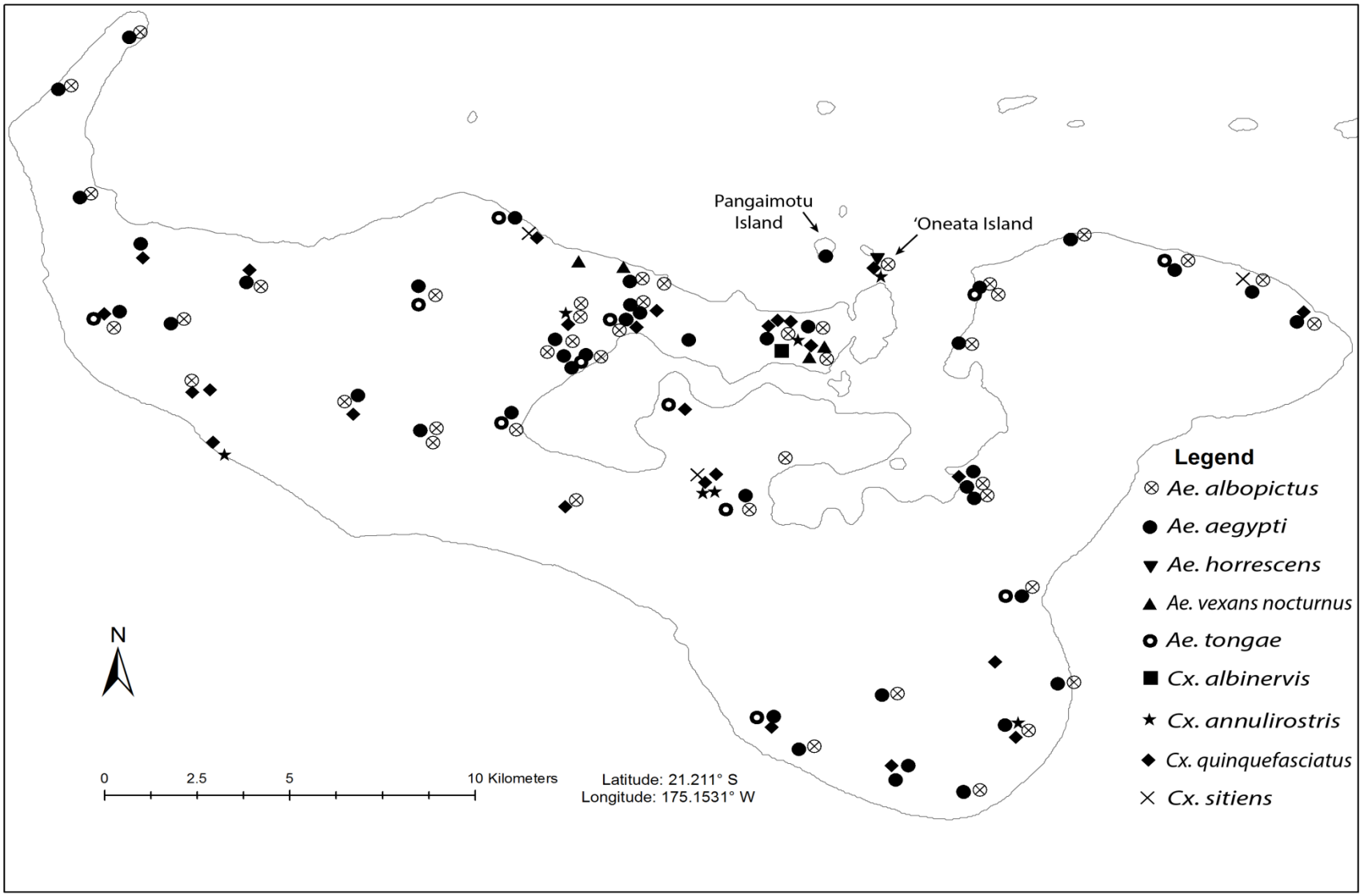


Figure 3.4: Distribution of mosquito species on Tongatapu, Pangaimotu and 'Oneata Islands in 2013 (n=68 sites on Tongatapu Island, 3 sites on 'Oneata Island and 1 site on Pangaimotu Island).

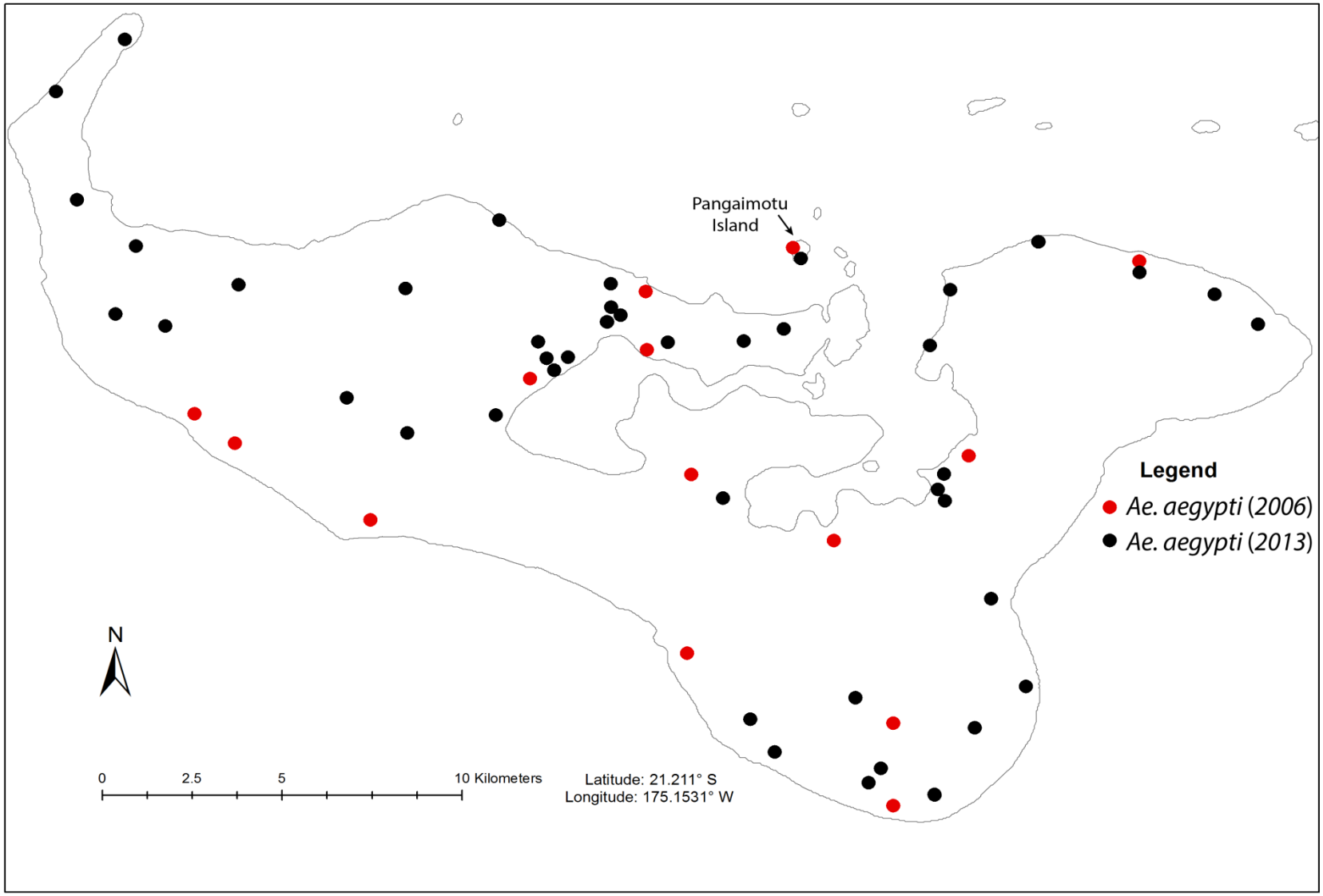


Figure 3.5: Distribution of *Ae. aegypti* on Tongatapu and Pangaimotu islands in 2006 (n=13 sites out of 32 sites sampled on Tongatapu Island and 1 site out of 1 site sampled on Pangaimotu Island) and 2013 (n=43 sites out of 68 sites sampled on Tongatapu Island and 1 site out of 1 site sampled on Pangaimotu Island).

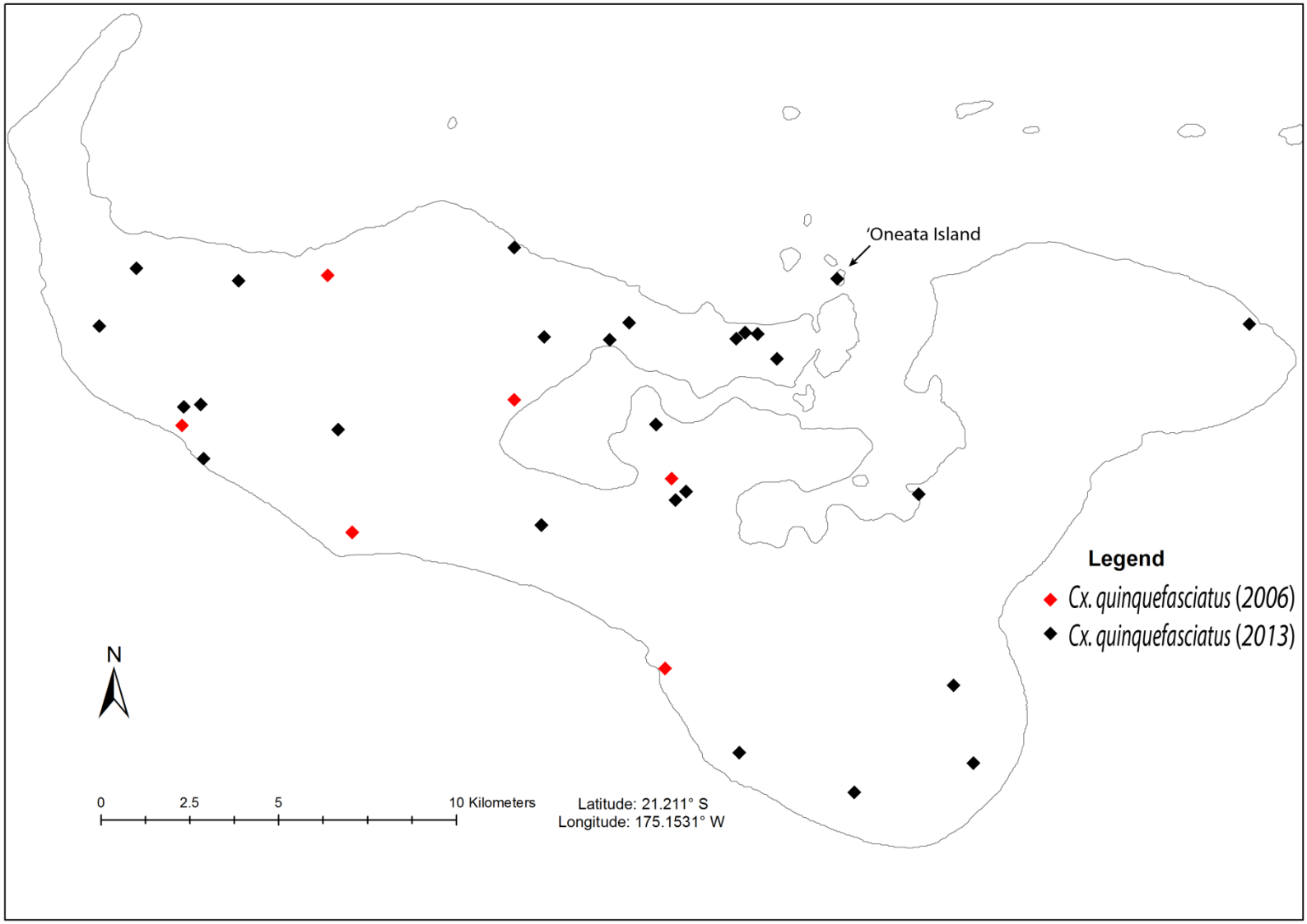


Figure 3.6: Distribution of *Cx. quinquefasciatus* on Tongatapu and 'Oneata islands in 2006 (n=6 sites out of 32 sites sampled on Tongatapu Island) and 2013 (n=24 sites out of 68 sites sampled on Tongatapu Island and 1 site out of 3 sites sampled on 'Oneata Island).

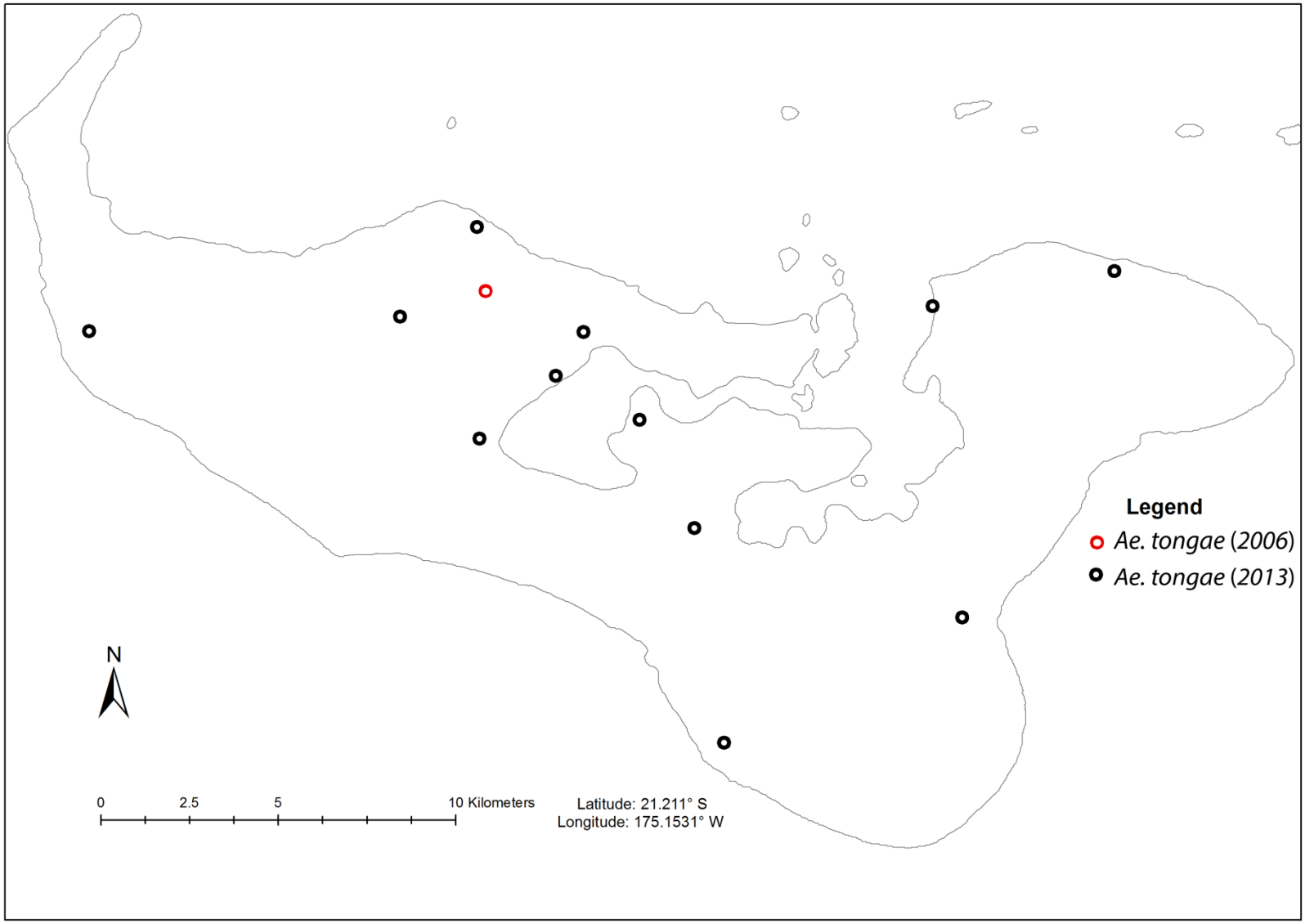


Figure 3.7: Distribution of *Ae. tongae* on Tongatapu Island in 2006 (n=1 site out of 32 sampled) and 2013 (n=12 sites out of 68 sites sampled).

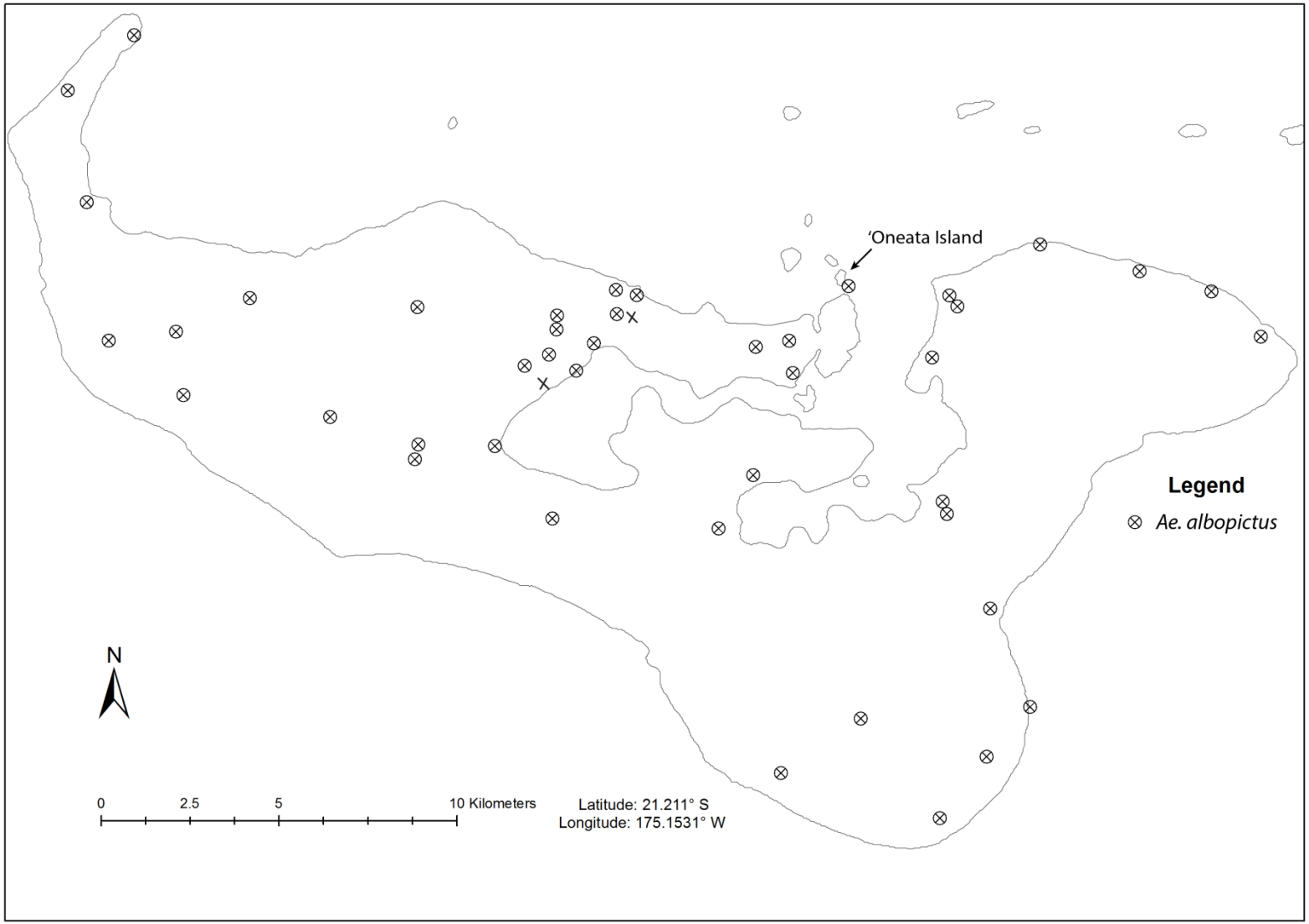


Figure 3.8: Distribution of *Ae. albopictus* on Tongatapu Island in 2013 (n=24 sites out of 68 sites sampled on Tongatapu Island and 1 site out of 3 sites sampled on 'Oneata Island). X marks the approximate locations that *Ae. albopictus* was first collected by Guillaumot et al. (2012).

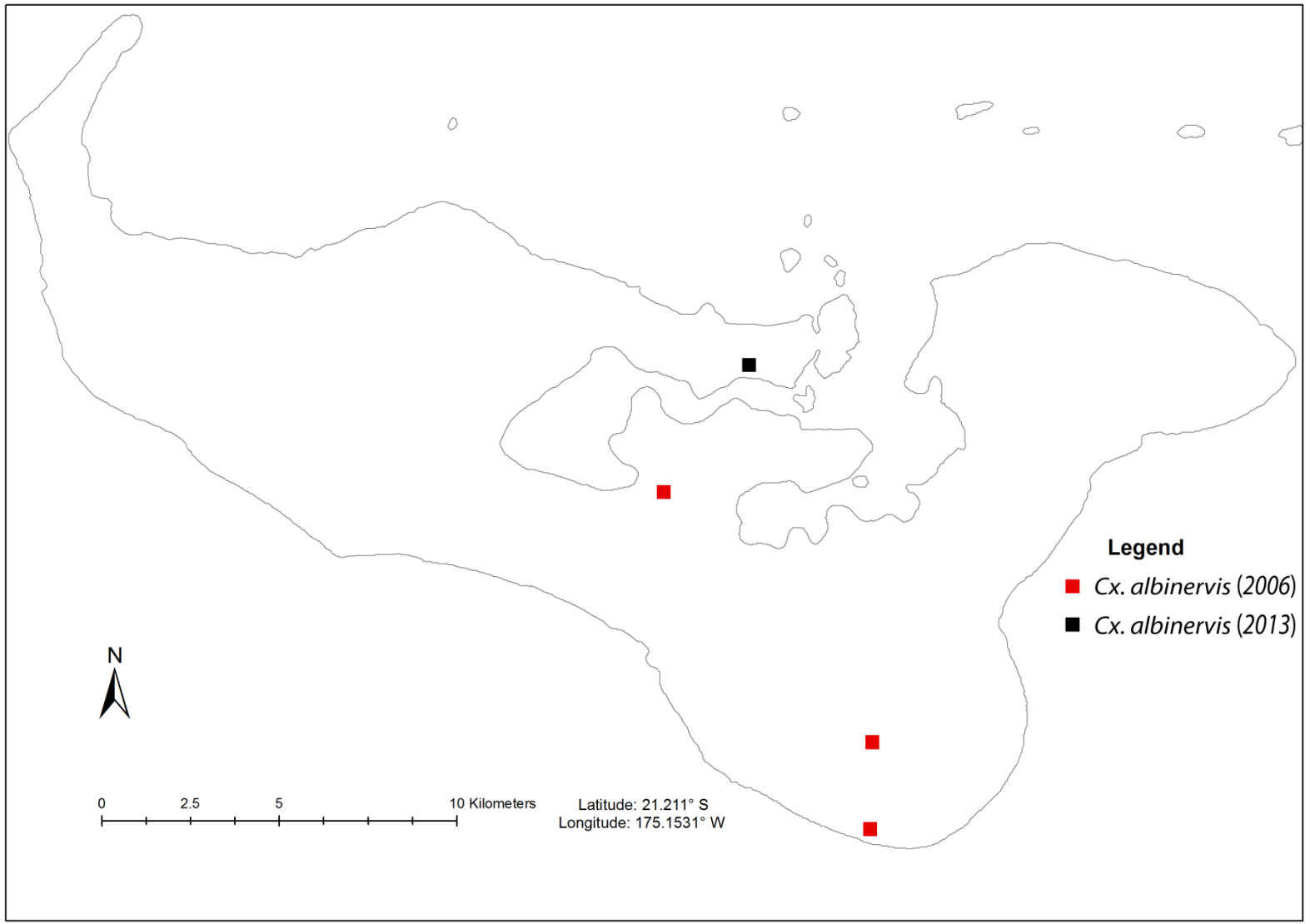


Figure 3.9: Distribution of *Cx. albinervis* on Tongatapu Island in 2006 (n=3 sites out of 32 sites sampled) and 2013 (n=1 site out of 68 sites sampled).

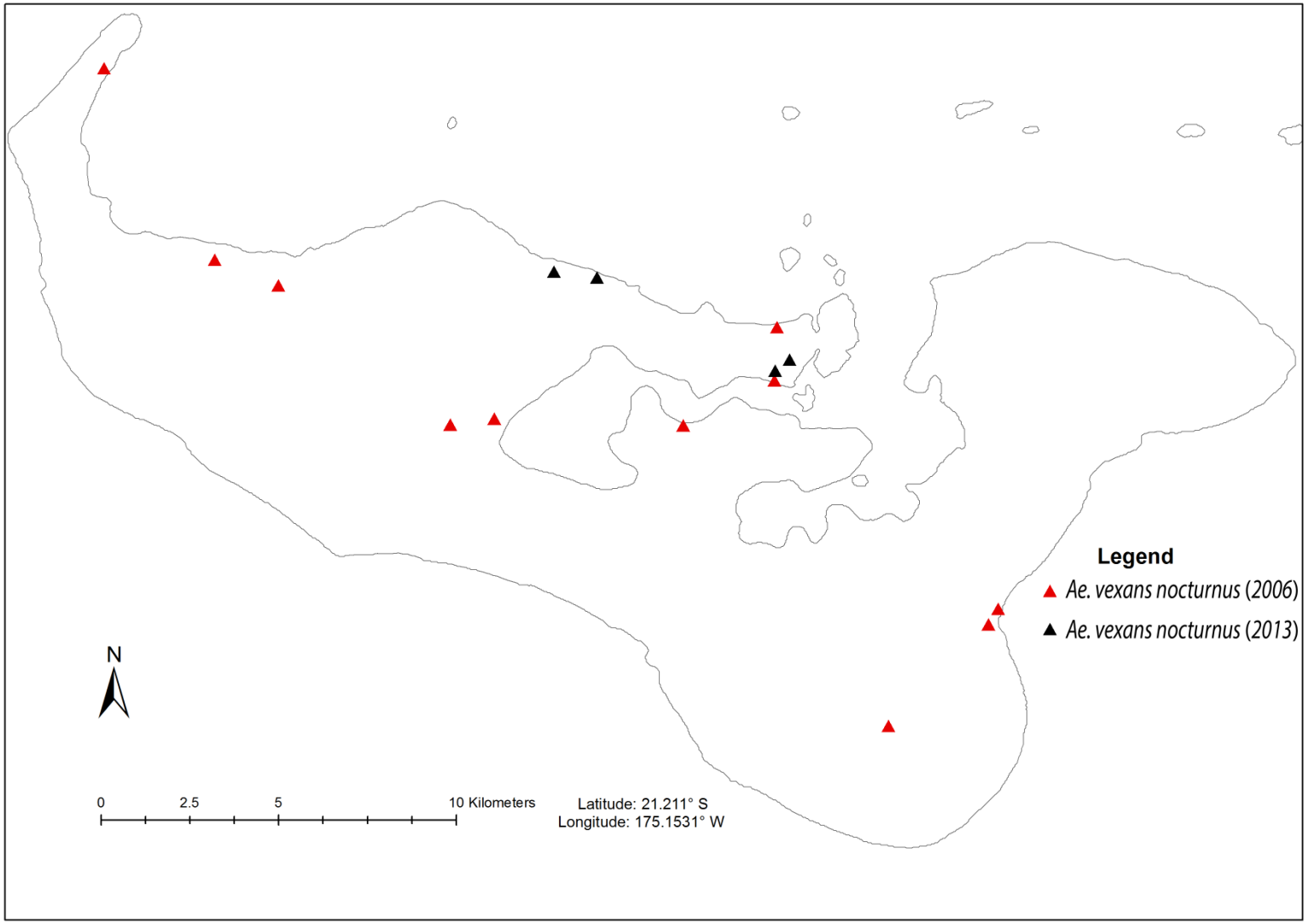


Figure 3.10: Distribution of *Ae. vexans nocturnus* on Tongatapu Island in 2006 (n=11 sites out of 32 sites sampled) and 2013 (n=4 sites out of 68 sites sampled).

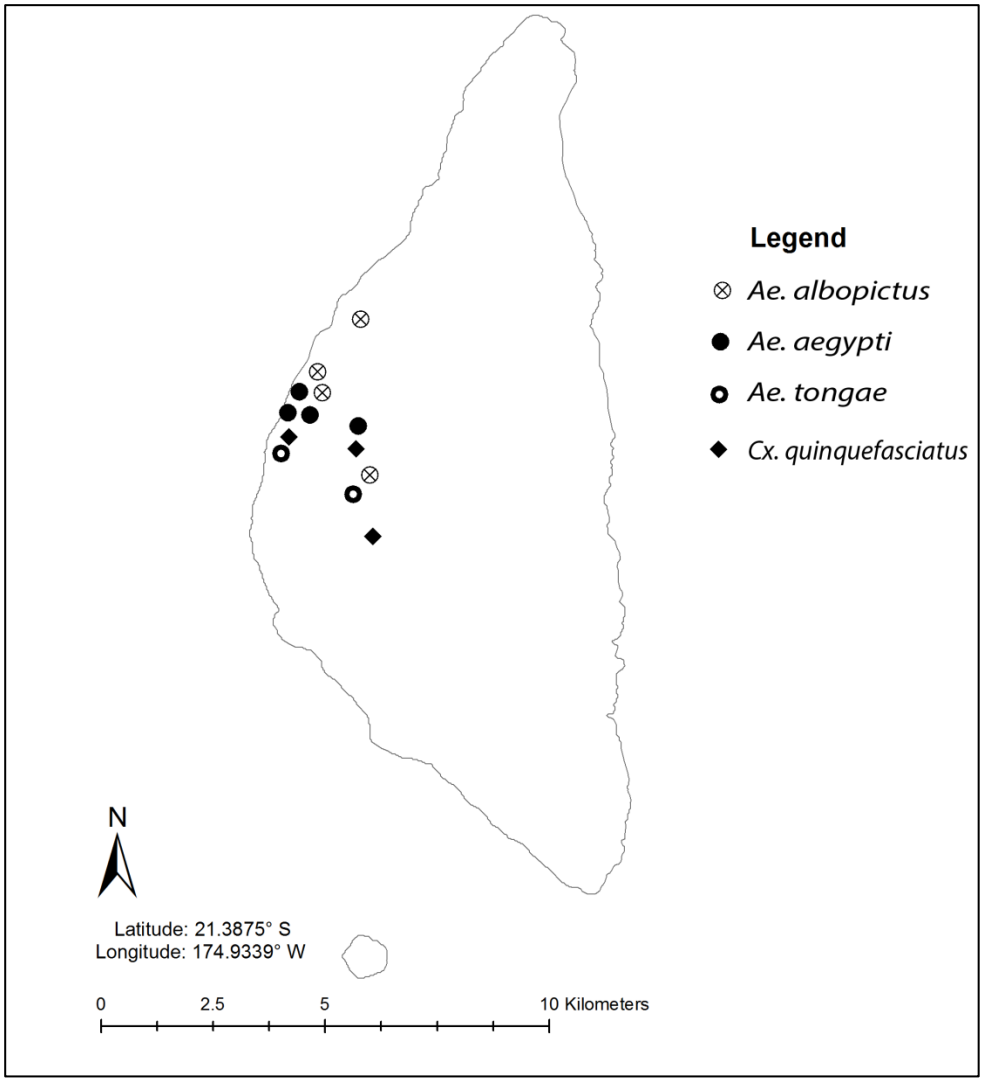


Figure 3.11: Distribution of mosquito species on ‘Eua Island (n=12 sites).

Discussion

Since 1926, 16 species of mosquito larvae have been observed in the Kingdom of Tonga. The 2013 survey confirmed the presence of nine mosquito species in the Tongatapu Island Group. It also confirmed the establishment and spread of *Ae. albopictus*, which may not have been present in 2006, and was first recorded in Tongatapu by Guillaumot et al. (2012). Interestingly, *Ae. albopictus* was collected on all four islands surveyed. The finding of this species is not surprising given the proximity of Tonga to Fiji where *Ae. albopictus* has been present since 1989 (Guillaumot et al. 2012). *Aedes albopictus* was most likely introduced to Tonga via sea and air traffic of passengers and goods between these countries, possibly as larvae or eggs in a car tyre, or as an adult transported in trade ships (Guillaumot et al. 2012). The international spread of car tyres has meditated the spread of *Ae. albopictus* globally (Reiter and Sprenger 1987; Benedict et al. 2007) as water held within them is an ideal habitat for mosquito eggs and larvae. The eggs of *Ae. albopictus* are drought-resistant, enabling months of survival if the water in the tyres dry out before reaching their destination and becoming inundated by water again (Enserink 2008). This trait may have aided its establishment in Tonga by allowing survival during periods of intensive drought.

In 1962 Belkin stated that "*Ae. albopictus* does not occur in the South Pacific. It is unlikely that it will become established, for it does not seem to be able to compete with other members of the *scutellaris* group (such as *Ae. hensilli*)". Belkin referred to the presence of *Ae. hensilli* on Yap Island, Micronesia preventing the establishment of *Ae. albopictus*. Additionally, the introduction of *Ae. albopictus* on a Tuamotu atoll in the 1970s in an attempt to outcompete *Ae. polynesiensis* failed to establish (Rosen et al. 1976). However, increased urbanisation and international trade between countries is likely to have brought about the introduction of *Ae. albopictus* to Tonga. The presence in Tonga of four other *Aedes* species belonging to the subgenus *Stegomyia* (*Ae. aegypti, Ae. tongae, Ae. horrescens, Ae. vexans nocturnus*) has not prevented *Ae. albopictus* from establishing itself.

The three species that Belkin (1962) suggests are endemic to Tonga (*Cx. sitiens, Cx. annulirostris*, *Ae. tongae*) were among the rarest of the species collected in my survey, with *Cx. sitiens* and *Cx. annulirostris* seeming to occur at very few sites in low numbers on both Tongatapu and 'Eua islands. Three introduced species, *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* were the most commonly collected mosquito species throughout the island group, probably because of the abundance of artificial habitats (particularly car tyres) which they commonly inhabited.

Aedes cooki, Ae. kesseli, and *Ae. tongae* have been shown to be closely related and may represent a single polymorphic species (Hoyer and Rozeboom 1977). They may therefore have been identified in earlier studies under different names (Table 3.1). *Aedes oceanicus* Belkin has not been collected in Tonga since at least 1956 (Table 3.1) (Laird 1956), however, due to its specific habitat preference (just above the water level in the leaf bases of *Colocasia* plants), it may have been difficult to collect and was subsequently not found in other larval surveys. Early observations of *Ae. oceanicus* in Samoa have alluded to a preference for natural habitats (e.g., axils of taro leaves), but little else is known about this species (Lambdin et al. 2008). Interestingly, *Ae. oceanicus* has been found occupying artificial habitats (e.g., used car tyres, buckets, cans, containers) in Samoa in recent years (Lambdin et al. 2008). Future surveys should be aware of the possibility that *Ae. oceanicus* may still occur in Tonga.

Harding et al. (2007) refer to *Ae. vexans nocturnus* being widely distributed and in high abundance throughout Tongatapu. However, it did not occur in large numbers in my survey and was only collected at four sites in Nuku'alofa and surrounding villages. Ramalingam (1976) also recorded *Ae. vexans nocturnus* in low numbers. This may possibly be an effect of competitive displacement from the more common species present throughout the Tongatapu Island Group (*Ae. aegypti*, *Ae. albopictus*, *Ae. tongae*, *Cx. quinquefasciatus*) which will be further discussed in Chapter Four.

Since the 2006 survey, the distribution of mosquitoes appears to have increased across Tonga, possibly for the following reasons. An increase in host availability for biting adults, stemming

from an increase in tourism in the islands; changes in climate that maybe affecting the lengths of dry and wet seasons; and an increase in refuse and car tyres, resulting in an increase of available habitats for larvae. A reduction in the number of car tyres available as breeding sites is likely to be one of the most practical ways of reducing mosquito numbers.

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Chapter Four: Habitat selection and species occurrence

Abstract

The occurrence of mosquito species and abiotic factors affecting their presence was investigated in the Tongatapu Island Group, Kingdom of Tonga. Mosquito larvae were collected at 84 sites with water chemistry measurements (dissolved oxygen, conductivity, pH, temperature) and habitat characteristics (length, width, depth) measured at each site. Species were collected across 23 different habitat types with artificial habitats (n=74) being more common than natural habitats (n=10). *Aedes aegypti* Linnaeus, *Ae. albopictus* Skuse, and *Culex quinquefasciatus* Say were the three most common species occurring at 59%, 53%, and 35% of all sites. *Ae. tongae* (Edwards) (16% of all sites), *Cx. annulirostris* Skuse (7% of all sites), *Ae. vexans nocturnus* Theobold (4% of all sites), *Cx. sitiens* Wiedemann (3% of all sites), *Cx. albinervis* Edwards (1% of all sites), and *Ae. horrescens* Edwards (1% of all sites) occurred less frequently. Co-occurrence of multiple species at the same site was common with two species co-occurring at 50% of all sites, and only a single species at 35% of sites (3 species at 13% of sites and 4 species at 2% of sites). The two most common co-occurring species were *Ae. aegypti* and *Ae. albopictus* (41.8% of all sites) and the three most common co-occurring species were *Ae. aegypti*, *Ae. albopictus*, and *Ae. tongae* (7.2% of all sites where two or more species occurred). Habitat volume was a significant predictor of species presence for *Ae. albopictus* (R^2 = 0.04, P = 0.02) and *Ae. tongae* (R^2 = 0.08, P = 0.00). Conductivity was a significant predictor of species presence for *Cx. annulirostris* (R^2 = 0.17, P = 0.02). Additionally, the volume by temperature interaction was a significant predictor of species presence for *Ae. aegypti* (R^2 = 0.04, P = 0.04), *Ae. albopictus* (R^2 = 0.05, P = 0.02) and *Cx. annulirostris* (R^2 = 0.29, P = 0.00). Finally, the volume by conductivity interaction was a significant predictor of species presence for *Ae. albopictus* (R^2 = 0.06, P = 0.01). The number of artificial habitats available may have significantly increased since

previous studies, and management should focus on reducing the number of habitats in order to control mosquito populations.

Introduction

Mosquitoes have complex life cycles with both aquatic and terrestrial stages. Female adults require water for oviposition. Mosquito eggs are laid on stagnant water using almost any available natural (e.g., pools, ponds, tree holes) and artificial (e.g., used car tyres, fuel drums, containers) habitat. The physicochemical characteristics of larval habitats are important determinants for the growth and development of immature mosquitoes (Mwangangi et al. 2007). Interactions between biotic (e.g., primary productivity, competition, predation) and abiotic (e.g., hydrology, temperature, pH, salinity, nutrient availability) variables are known to influence larval densities (Washburn 1995; Stresman 2010; Rejmánková et al. 2013). For example, warmer water temperatures can decrease the duration of larval development from egg to adult (Tun-Lin et al. 2000).

Research by Mogi (1981) and Washburn (1995) has shown that predation can regulate mosquitoes in natural habitats (in this study pools), whereas mosquitoes in artificial habitats are more likely to be limited by resources than predation. Predatory fish (*Poecillia mexicana* Steindachner, and *Gambusia affinis* Baird and Girard), and insects, such as water boatmen *Corixa punctata* Illiger, and dragonfly nymphs (*Bradinopyga geminata* Rambur, and *Ceriagrion coromandelianum* Fabricius) commonly occur in natural habitats and prey on mosquito larvae. In artificial habitats such predators are usually absent (Service 1977; Collins and Washino 1985; Quiroz-Martínez and Rodríguez-Castro 2007; Venkatesh and Tyagi 2013). Their absence is likely a consequence of habitat size and the inability of larger predators to colonise smaller habitats (e.g., used car tyres, fuel drums). This is likely to affect the community structure of mosquito communities occurring in smaller habitats. For example, Sunahara et al. (2002) found that *Aedes* (Stegomyia) species rarely co-occurred with predators in small containers (<0.1 m^2), allowing for widespread mosquito occurrence in these smaller habitats.

The climate of islands in the South Pacific is tropical across all seasons, punctuated by distinctive wet and dry seasons. Island countries closer to the equator (e.g., Solomon Islands, Tuvalu, Samoa) have warmer average temperatures, than countries further away from it (e.g., Cook Islands, Fiji, Tonga, New Caledonia) (South Pacific Weather 2015). Extreme weather events, such as tropical cyclones often occur from April–November each year (South Pacific Weather 2015) and are known to be associated with mosquito outbreaks by inundating larval habitats (Carrington et al. 2013; Chaves et al. 2014).

Tonga has distinctive wet (November–April) and dry seasons (May–October) (Australian Bureau of Meteorology 2011). Annual rainfall ranges from 1,780–2,340 mm (Thompson 1986), and results in regular formation and inundation of temporary natural pools, ideal for mosquito colonisation. Despite attempts to reduce mosquito numbers, their persistence in Tonga is maintained because of several factors, such as; poor refuse management and the importation of used car tyres, which provides a plethora of artificial habitats for larval colonisation.

In Tonga, it appears that the larvae of few mosquito species co-exist in the same habitat (Harding et al. 2007), however, factors that influence this low frequency of co-occurrence have not been studied. In this chapter I examine whether habitat type, size, and abiotic and biotic variables can explain differences in patterns of mosquito co-occurrence and community assembly.

Materials and methods

Mosquito sampling

I sampled 84 sites throughout the Tongatapu Island Group. Within each sampling area, potential larval habitats were searched for in a 300 m circumference. A site is defined as a location in which sampling occurred from a single habitat in any given village. No more than two sites were collected from within each 300 m circumference. If no habitats were found, local inhabitants were asked

about possible larval habitats outside of it. At each site a range of habitat conditions were recorded including habitat wetted length (cm), width (cm), and depth (cm) using a tape measure. Dissolved oxygen and temperature were measured using a YSI 550a water quality meter, and specific conductivity and pH were measured using an Oakton pH/CON 10 meter. Larvae were sampled with mosquito dippers, turkey basters and pipettes. Samples were preserved in the field in 50 ml vials of 70% ethanol. Vials were sealed with Whatman® Laboratory Sealing Film and returned to the University of Canterbury, New Zealand for laboratory identification.

Volume calculations

The volume of habitats was calculated using the equation: length x width x depth

However, the volume associated with car tyres was calculated by subtracting one volume from another using the formula: πR^2 - πr^2. A worked example is shown in Appendix 4.1.

Statistical analyses

Data were entered in Microsoft Excel and all analyses were performed in RStudio (v0.98, R version). Mosquito presence was recorded as binomial data (presence/absence) for analyses. Sites were categorised into 23 different habitat types for analyses. Percentage bar graphs were used to analyse the preference of mosquito species in relation to habitat type. Pearson's correlation coefficients were used to determine associations among environmental variables. Model simplification was based on significance, employing stepwise backwards selection procedures based on AIC values. Variables which were not significant were excluded from the model. The degree of association between mosquito species was examined with chi-squared tests. Generalised Linear Models fitting logistic regression models were used to test the association between environmental variables for explaining the presence of mosquito species.

Results

Mosquitoes were readily collected in a wide range of habitats. Of 84 sites, 74 were artificial, and 10 were natural habitats. In total, mosquitoes were collected from 23 different habitat types (Appendix

4.2). A total of nine mosquito species were found, many of which co-occurred at the same sites. *Ae. aegypti* was the most common species occurring at 50 sites (59% of all sites). *Ae. albopictus* occurred at 45 sites (53% of all sites), while *Cx. quinquefasciatus* was the third most common species occurring at 30 sites (35% of all sites). *Ae. tongae* was found at 14 sites (16% of all sites), *Cx. annulirostris* at 6 sites (7% of all sites), *Ae.vexans nocturnus* at 4 sites (4% of all sites), *Cx. sitiens* at 3 sites (3% of all sites) and *Cx. albinervis* and *Ae. horrescens* at a single site.

Natural and artificial habitats

72% of *Aedes aegypti* larvae occurred in artificial habitats, and 28% in natural habitats sampled (Figure 4.1), whereas *Ae. albopictus* occurred predominantly in artificial (82%) rather than natural (18%) habitats (Figure 4.1). In contrast, *Cx. quinquefasciatus* occurred in 37% of artificial habitats, and in 63% of natural habitats (Figure 4.1). *Aedes tongae* was found in 40% of artificial and 60% of natural habitats and *Cx. sitiens* was found in 18% of artificial and 82% of natural habitats (Figure 4.1). *Aedes vexans nocturnus* and *Cx. albinervis* were both rare and only occurred in natural habitats (Figure 4.1). In contrast, *Cx. annulirostris* and *Ae. horrescens* were also rare and found only in artificial habitats (Figure 4.1).

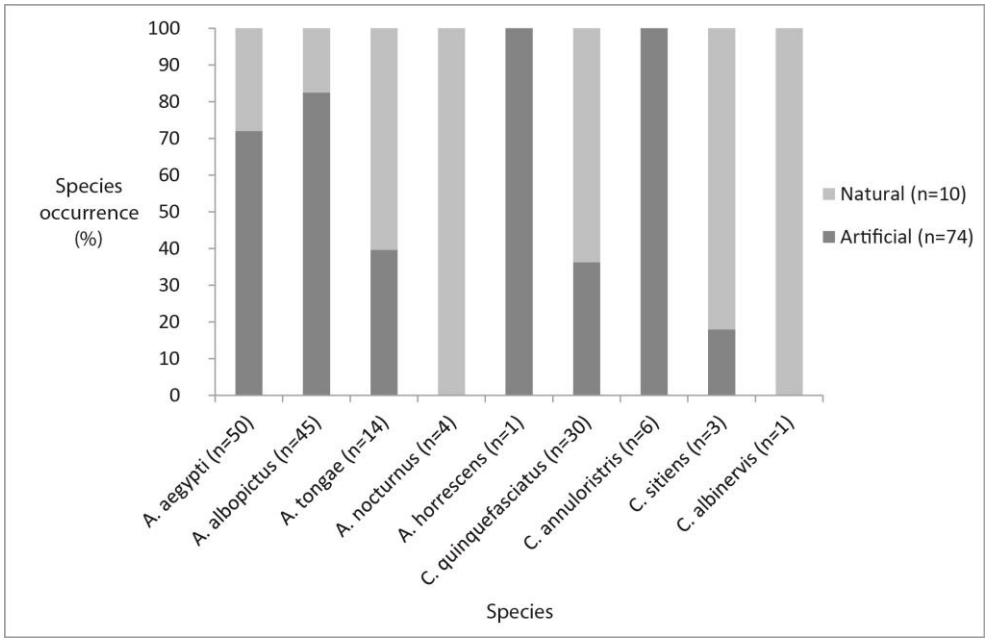

Figure 4.1: Species occurrence in artificial and natural habitats (n=84) based on presence/absence of species at each site.

Habitat selection

Car tyres, water containers, fuel drums, ponds, fridges, and washing machines were the most common habitat types (Figure 4.2, 4.3, Appendix 4.2) with the three most common mosquito species using all of these habitats (Figure 4.2). *Aedes aegypti* occurred most commonly in artificial habitats, occurring in 42% of car tyres, water containers, and fuel drums, 16% of fridges, and 38% of washing machines examined (Figure 4.2). *Aedes albopictus* was also found most commonly in artificial habitats, occurring in 28% of car tyres and water containers, 33% in fridges, and 25% in washing machines (Figure 4.2). *Culex quinquefasciatus* also used a variety of artificial and natural habitats, occurring in 43% of fuel drums, 33% of fridges and ponds, 25% of washing machines, 14% of water containers, and 9% of car tyres (Figure 4.2). *Aedes tongae* occurred in 14% of fuel drums, 17% of fridges, 11% of car tyres, and 13% of washing machines (Figure 4.2).

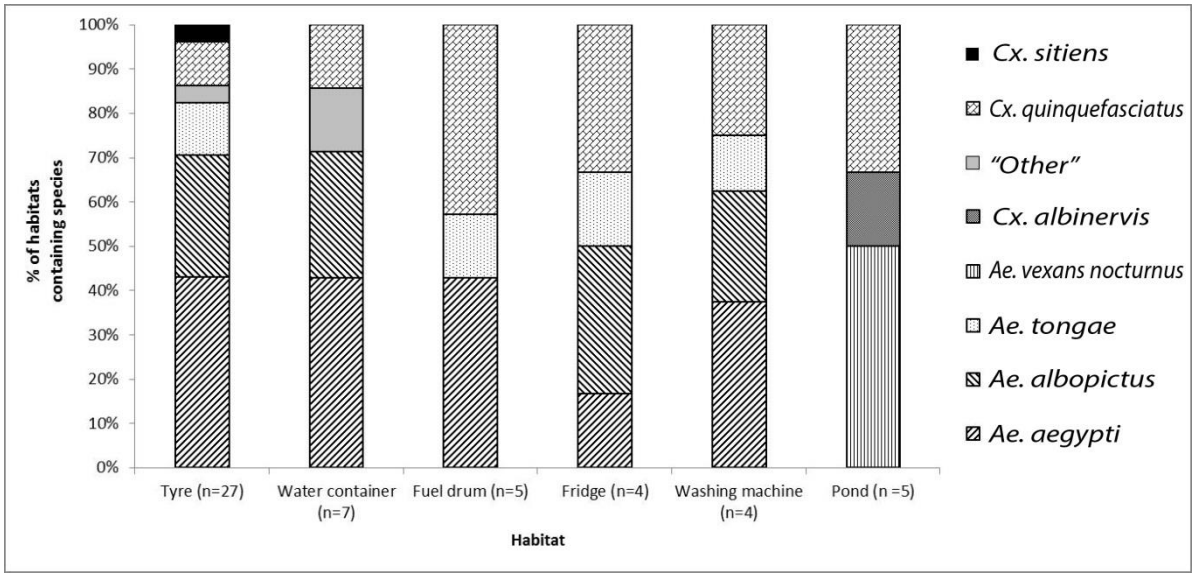

Figure 4.2: Habitat occurrence by species of mosquito species in the 6 most commonly inhabited habitats across 84 sites. Artificial habitats: car tyre, water container, fuel drum, fridge, washing machine. Natural habitats: pond. "Other" refers to *Ae. horrescens* and *Cx. annulirostris* species.

Figure 4.3: The six most common mosquito habitat-types sampled in the Tongatapu Island group. (a) a car tyre, (b) a bucket, (c) a fuel drum, (d) a freezer, (e) a washing machine, (f) represents a natural pond habitat.

Co-occurrence of species

Co-occurrence of larvae of multiple species in the same habitat was relatively common with two species co-occurring in 50% of study sites (n= 42 sites), and only a single species in 35% of sites (n= 29 sites). Occurrence of three or more species was less common with 3 species co-occurring in 13% of sites (n= 11 sites) and 4 species in 2% of sites (n= 2 sites) (Appendix 4.3).

In sites with only single species occurrences, *Ae. aegypti* was found alone in 24% of sites (n=7 out of 29 sites where a single species occurred), *Ae. albopictus* was found alone in 28% of sites (n=8 out of 29 sites where a single species occurred) (Figure 4.4), *Cx. quinquefasciatus* was found

alone in 28% of sites (n=8 out of 29 sites where a single species occurred) (Figure 4.5) and *Ae. tongae* was found alone in 3% of sites (n=1 out of 29 sites where a single species occurred) (Figure 4.6).

The two species co-occurring most commonly were *Ae. aegypti* and *Ae. albopictus* at 55% of sites (n= 23 out of 42 sites where two species co-occurred) (Figure 4.4). *Ae. aegypti* and *Cx. quinquefasciatus* co-occurred in 14% of all sites where co-existence of 2 or more species occurred (n= 6 out of 42 sites where two species co-occurred) (Figure 4.5), whilst *Ae. aegypti* and *Ae. tongae* co-occurred in 7% of all sites (n= 3 out of 42 sites where two species co-occurred)(Figure 4.6). The three most common co-occurring species, *Ae. aegypti*, *Ae. albopictus,* and *Ae. tongae* were found together in 36% of all sites where three species co-occurred (n= 4 out of 11 sites where three species co-occurred (Figure 4.6).

Chi-square analyses showed that *Ae. aegypti* were more likely to co-exist with *Ae. albopictus* than by chance alone ($\chi^2 = 5.444$, $P < 0.05$). Conversely, *Ae. albopictus* is less likely to co-exist with *Cx. quinquefasciatus* than by chance alone ($\chi^2 = 17.800$, $P < 0.01$).

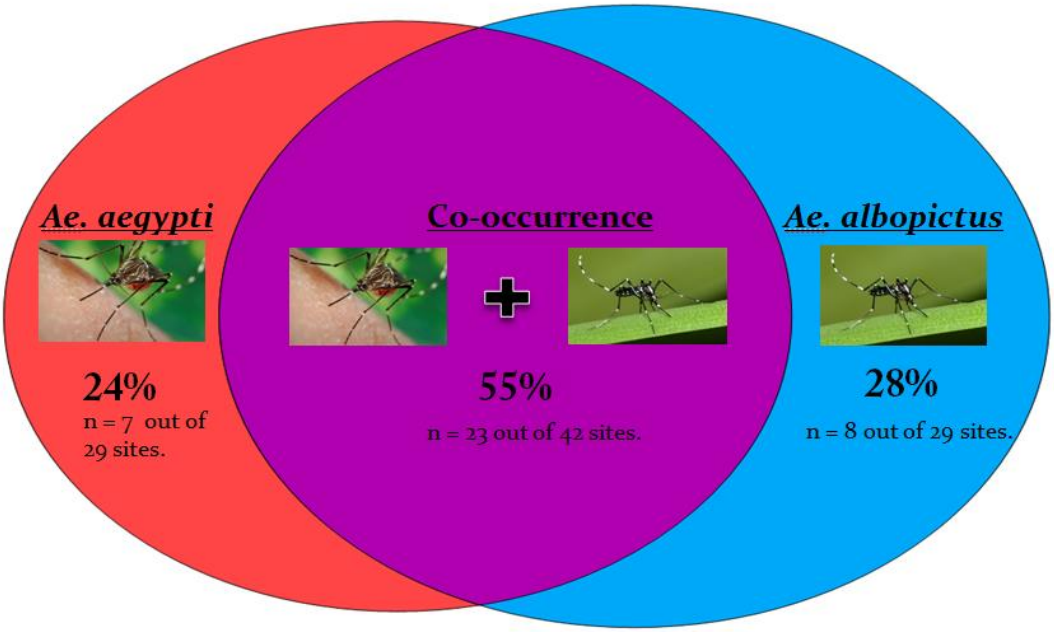

Figure 4.4: Comparison of the individual occurrence of *Ae. aegypti* and *Ae. albopictus* and co-occurrence of these two species in the same habitat type. Circles are proportional to relative percentage. Percentages do not equal 100%, due to omission of co-occurrence values with other species (see Appendix 4.3 for complete data).

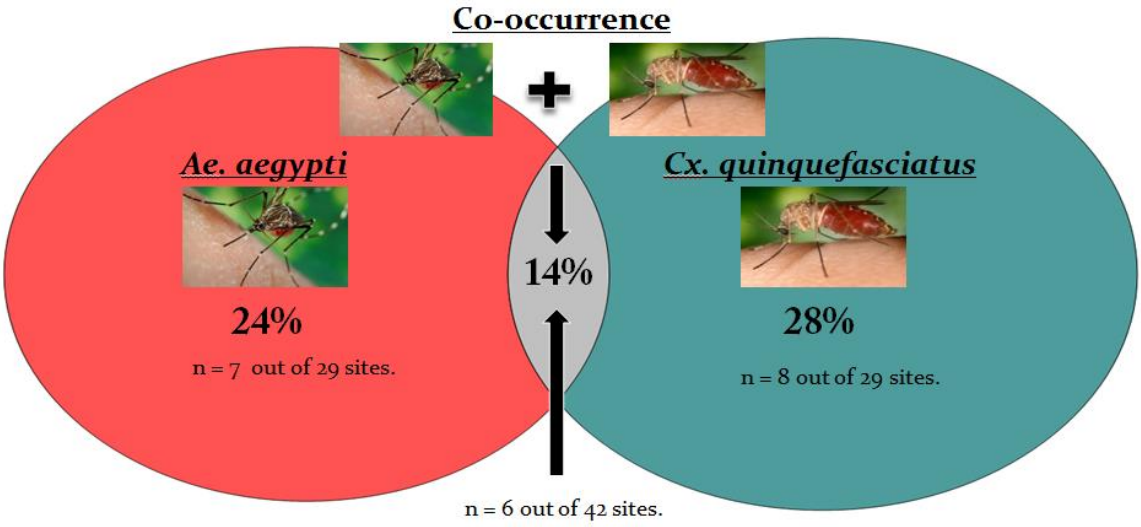

Figure 4.5: Comparison of the individual occurrence of *Ae. aegypti* and Cx. *quinquefasciatus* and co-occurrence of these two species in the same habitat type. Circles are proportional to relative percentage. Percentages do not equal 100 due to co-occurrence values with other species not shown here (see Appendix 4.3 for complete data).

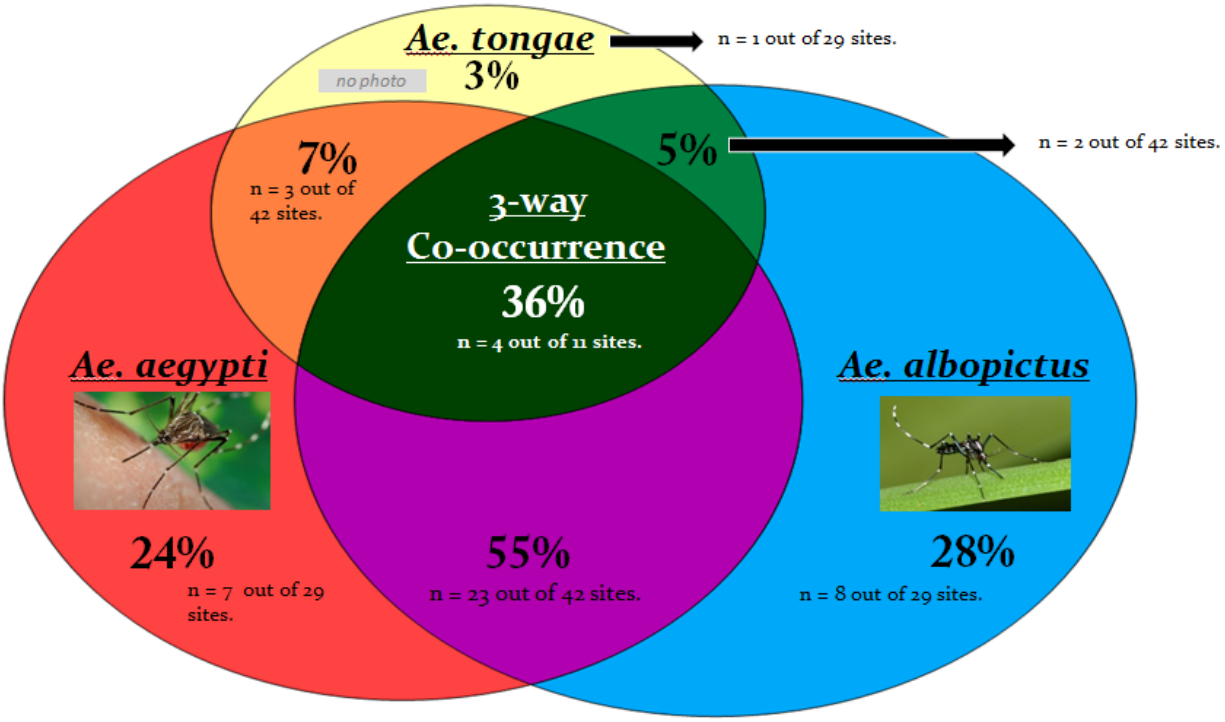

Figure 4.6: Comparison of the individual occurrence of *Ae. aegypti*, *Ae. tongae* and *Ae. albopictus* and co-occurrence of these three species in the same habitat type. Circles are proportional to relative percentage. Percentages do not equal 100 due to co-occurrence values with other species not shown here (see Appendix 4.3 for complete data).

Association between species and environmental variables

Pearson's correlation coefficients were used to examine relationships among six abiotic factors (Table 4.1): temperature, pH, dissolved oxygen, conductivity, volume, and depth. Several of the correlations were statistically significant (Table 4.1). Volume was positively correlated with depth ($r = 0.42$, $P < 0.01$) and volume was negatively correlated with dissolved oxygen concentration ($r = -0.27$, $P < 0.01$). Temperature was positively correlated with dissolved oxygen ($r = 0.23$, $P < 0.05$) and pH ($r = 0.23$, $P < 0.05$) and dissolved oxygen was positively correlated with pH ($r = 0.48$, $P < 0.01$) (Table 4.1). Dissolved oxygen, pH and depth were removed from GLM analyses due to multicollinearity. Model simplification identified which of the abiotic factors could best explain the presence of the four most common species. Thus, logistic regression models identified volume as a significant predictor of *Ae. albopictus* (coefficient = 21.9, $R^2 = 0.04$, $P < 0.05$) and *Ae. tongae* presence (coefficient = -22.6, $R^2 = 0.08$, $P = <0.01$) (Table 4.2) and conductivity was a significant

predictor of *Cx. annulirostris* presence (coefficient = 0.002, R^2 = 0.17, P < 0.05) (Table 4.2). Additionally, logistic regression models identified a significant interaction between volume and temperature (coefficient = -0.12) for *Ae. aegypti* (R^2 = 0.04, P < 0.05). As volume increases (coefficient = 3.42) the effect of temperature decreases (coefficient = 0.03). The same effect was true for *Ae. albopictus* (R^2 = 0.05, P < 0.05) (volume coefficient = 21.9, temperature coefficient = 0.18, volume and temperature coefficient = -0.73) and *Cx. annulirostris* (R^2 = 0.29, P < 0.01) (volume coefficient = 5.27, temperature coefficient = 7.33, volume and temperature coefficient = -1.96). Furthermore, logistic regression models identified a significant interaction between volume and conductivity (coefficient = -0.01) for *Ae. albopictus*. As volume increases (coefficient = 21.9), the effect of conductivity decreases (coefficient = 0.01).

Logistic regression models were also used to predict the probable presence of a species under certain abiotic conditions. *Ae. albopictus* occurred more frequently in habitats with a volume > 0.01m^3 (Figure 4.7), while *Ae. tongae* was only found in habitats with a volume < 0.1m^3 (Figure 4.8). Habitats in which *Cx. annulirostris* was found varied in conductivity, from 70 µS cm^{-1} to 1500 µS cm^{-1} (minimum = 30.2 µS cm^{-1}, maximum = 1848, mean = 543) (Figure 4.9).

Table 4.1: Pearson's product-moment correlation coefficients between six environmental variables from 84 survey sites. Correlation variables scaled from -1 to 1 and displayed with two decimal places. Statistically significant values are indicated with *, P < 0.05; **, P<0.01; ***, P < 0.001.

Factors	Temperature (°C)	Dissolved Oxygen (mgL^{-1})	pH	Conductivity ($\mu S\ cm^{-1}$)	Depth (m)	Volume (m^3)
Temperature (°C)						
Dissolved Oxygen (mgL^{-1})	0.23* (p=0.03)					
pH	0.23* (p=0.04)	0.48*** (p=0.00)				
Conductivity ($\mu S\ cm^{-1}$)	-0.19	-0.08	-0.10			
Depth (m)	-0.08	0.08	0.16	-0.14		
Volume (m^3)	0.03	-0.27* (p=0.01)	-0.04	-0.07	0.42*** (p=0.00)	

Table 4.2: Logistic regression models showing effect of abiotic factors on mosquito species presence for 84 sites in the Tongatapu Island Group. Interaction effects are shown with a colon (:). Only factors that are statistically significant are shown. Statistically significant figures are indicated with *, P < 0.05; **, P<0.01; ***, P < 0.001. *Aedes horrescens* (n=1)*, Cx. albinervis* (n=1), *Cx. sitiens* (n=3) and *Ae. vexans nocturnus* (n=4) were excluded due to low occurrence of sampling. Coefficients of determination (R^2), p values were calculated for each variable as terms were entered last in the model (Type II analysis of deviance).

Species	**Abiotic factors**	**df**	***R^2***	**p value**
Ae. aegypti (n = 50)	Volume:Temperature	80	0.04	<0.05*
Ae. albopictus (n = 45)	Volume Volume:Temperature Volume:Conductivity	77 77 77	0.04 0.05 0.06	<0.05* <0.05* <0.01**
Ae. tongae (n = 14)	Volume	82	0.12	<0.01**
Cx. annulirostris (n = 6)	Conductivity Volume:Temperature	79 79	0.17 0.29	<0.05* <0.001***

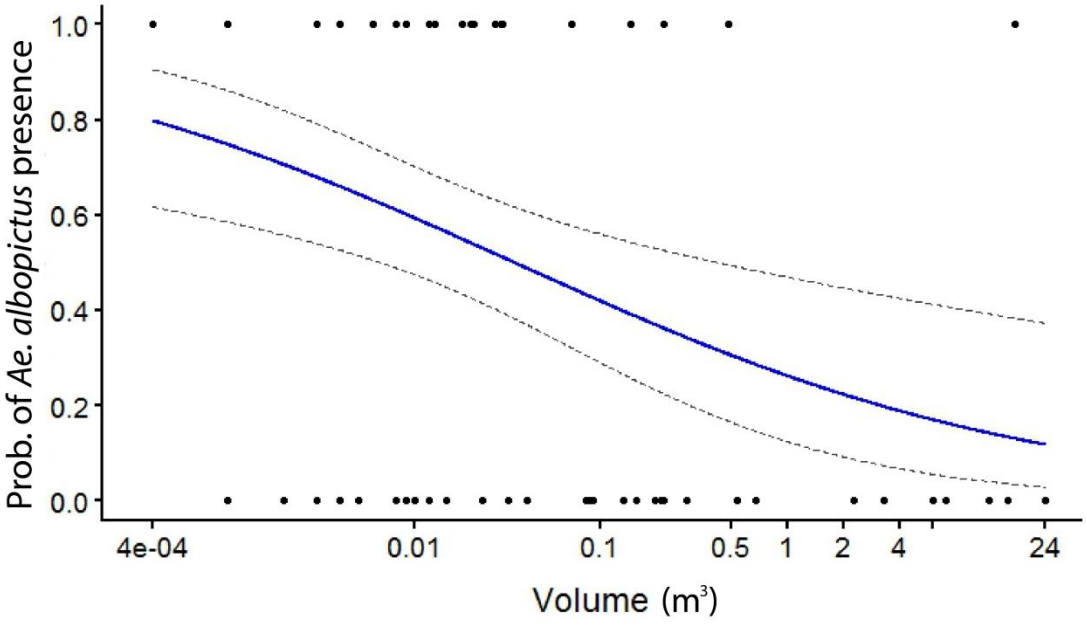

Figure 4.7: Logistic regression plot of the modelled probability of *Ae. albopictus* (n=45) presence with regard to volume for 84 sites in the Tongatapu Island Group. Only 19 points are shown on the 1.0 line as some points are overlapping on the same point (same volume value). Blue line is the line of best fit. Dashed lines represent the 95% confidence interval.

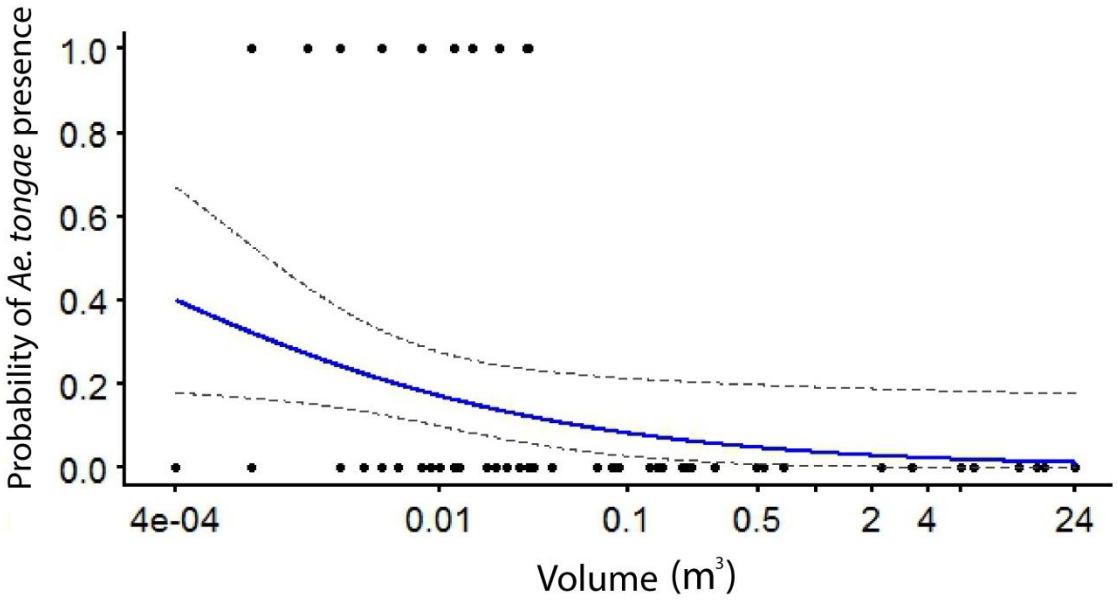

Figure 4.8: Logistic regression plot of the modelled probability of *Ae. tongae* (n=14) presence with regard to volume for 84 sites in the Tongatapu Island Group. Only 9 points are shown on the 1.0 line as some points are overlapping on the same point (same volume value). Blue line is the trend line. Dashed lines represent the 95% confidence interval.

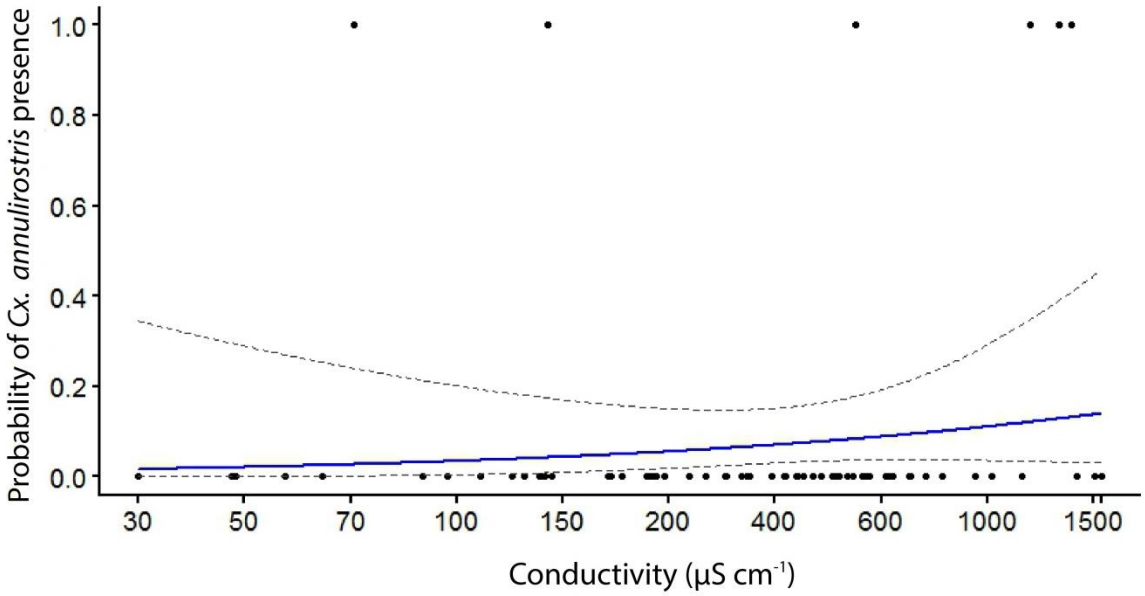

Figure 4.9: Logistic regression plot of the modelled probability of *Cx. annulirostris* (n=6) presence with regard to conductivity for 84 sites in the Tongatapu Island Group. Blue line is the trend line. Dashed lines represent the 95% confidence interval.

Discussion

Mosquito larvae inhabit a diverse range of artificial and natural habitats in the Tongatapu Island Group. In my survey, artificial habitats were predominant, accounting for 88% of all larval habitats. The importance of artificial habitats for many species further indicates that humans have facilitated the spread within the archipelago. Consistent with findings here, Harding et al. (2007) reported that car tyres, concrete water tanks, and 44-gallon drums were the most common habitats. The importance of these artificial habitats was particularly apparent for *Ae. aegypti* and *Ae. albopictus* in this study. In fact, the transportation of used car tyres from nearby islands may have facilitated the spread of *Ae. aegypti* and more recently *Ae. albopictus* into Tonga (Guillaumot et al. 2012). This has also been suggested as the primary vector for invasion into other countries for both these species (Reiter and Sprenger 1987; Benedict et al. 2007). In contrast, natural habitats accounted for only 12% of all larval habitats sampled. Given that random sampling in villages occurred, this suggests that *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* are preadapted to artificial habitats. *Aedes tongae*, *Ae. vexans nocturnus*, *Cx. albinervis*, *Cx. quinquefasciatus*, and *Cx. sitiens* were most commonly found in ponds and pools but these habitats were very rare throughout the Tongatapu

Island Group. Interestingly, *Ae. tongae* and *Cx. quinquefasciatus* were found in both artificial and natural habitats. This is contrary to the findings of Harding et al. (2007) in which *Cx. quinquefasciatus* and *Ae. tongae* were only found in artificial habitats; however, my findings are consistent with those of Lee et al. (1982) who noted that *Cx. quinquefasciatus* readily occurs in both natural and artificial habitats near human habitation in Australia. *Aedes* vexans *nocturnus*, *Ae. horrescens, Cx. albinervis, Cx. sitiens* and *Cx. annulirostris* were all found infrequently, possibly as a result of intraspecific competition resulting in exclusion by the more dominant species (*Ae. aegypti, Ae. albopictus, Ae. tongae*, and *Cx. quinquefasciatus*).

My survey was conducted over a single, particularly dry, wet season. Further research should focus on sampling both in the wet (November–May) and the dry (May–November) seasons to tease apart climatic factors on species occurrence.

Co-occurrence of species

In my survey, 50% of the 84 sites had two species co-occurring, whereas only 16% of the 42 sites surveyed by Harding et al. (2007) had two species co-occurring. Species which co-occurred in the Harding et al. (2007) study were; *Ae. vexans nocturnus, Ae. aegypti*, *Ae. horrescens, Cx. quinquefasciatus*, *Cx. annulirostris*, and *Cx. sitiens*. In my study, *Ae. vexans nocturnus* was not found in the presence of other species, however, it was only collected at four sites. Contrary to the findings of Harding et al. (2007), *Ae. aegypti* was not commonly found at the same sites with *Cx. quinquefasciatus*. In fact, *Ae. aegypti* more commonly co-occurred with *Ae. albopictus*.

The change in patterns of co-occurrence may be a result of the introduction of *Ae. albopictus* into Tonga. *Aedes albopictus* is known to be a competitively dominant species, with many examples of competitive exclusion having been reported (Juliano and Lounibos 2005). Competitive exclusion is based on the principle that different species cannot simultaneously occupy the same niche (Gause 1934; DeBach 1966) and in Europe, invasions of *Ae. albopictus* are likely associated with declines in the abundance of *Ae. aegypti* (Simberloff and Gibbons 2004). Additionally, in

Mayotte (Indian Ocean), *Ae. albopictus* appears to be competitively displacing resident *Ae. aegypti* species due to interspecific competition for resources in habitats occupied by the two species (Bagny et al. 2009). Consequently, *Ae. albopictus* has greatly expanded its distribution range in Mayotte (Bagny et al. 2009). Further afield however, the opposite is true as populations of *Ae. albopictus* have been displaced by *Ae. aegypti* in Brazil (Braks et al. 2003), Florida, USA (O'Meara et al. 1995; Braks et al. 2003), and Puerto Rico (Cox et al. 2007). Findings from these studies suggest the destruction of rural habitats and increases in urbanisation probably favour *Ae. aegypti*. However, this does not appear to be the case in Tonga where urban expansion is occurring, and *Ae. albopictus* appears to be established and dominant throughout the Tongatapu Island Group. This may be the result of intensified urbanisation, increasing the number of larval habitats that *Ae. albopictus* can colonise (particularly car tyres, and fuel drums) as has been shown in China (Li et al. 2014). Mechanisms behind competitive displacement may be driven by egg hatching inhibition, oviposition deterrence and larval resource or interference competition (Reitz and Trumble 2002). It is not known for how long *Ae. albopictus* has been present in Tonga, but future research should focus on distinctive "rural" and "urban" mosquito habitats where *Ae. albopictus* and *Ae. aegypti* co-occur, and the mechanisms driving single species occurrences.

Importance of environmental variables in determining species occurrence

Habitat volume was a significant predictor of the presence of *Ae. albopictus* and *Ae. tongae* with both species being collected in natural and artificial habitats. Interestingly, logistic regression models predicted *Ae. albopictus* presence in habitats with larger volume, whereas logistic regression models predicted *Ae. tongae* presence in habitats with smaller volume. This may be due to environmentally dependent competitive exclusion of *Ae. tongae* by *Ae. albopictus*. For instance, in larger volume habitats *Ae. albopictus* may competitively exclude *Ae. tongae.* However, smaller volume habitats may be more drought prone, which physiologically exclude *Ae. albopictus* allowing *Ae. tongae* to occur.

This is further supported by the low frequency of co-occurrence between these species. Similar mechanisms have been shown to drive species distributions in fish (Mcintosh 2000; White et al. 2014) and invertebrate community assemblage patterns in experimental drought mesocosms (Chase et al. 2007). However this result seems somewhat contradictory to what is already known about the physiological trait of *Ae. albopictus* in tolerating drought-like conditions (Enserink 2008). It may be possible that *Ae. tongae* has a similar physiological trait, or that another factor not tested in this research could be important (e.g., physiological limits of these species, conspecific effects). An experimental drought mesocosm experiment, similar to what Chase et al. (2007) completed would be interesting to conduct to tease apart these factors.

Research by Vezzani and Schweigmann (2002) has shown that oviposition site selection for species preferring to colonise containers (*Ae. aegypti, Ae. albopictus*, *Ae. tongae*, *Cx. quinquefasciatus* in this instance) depends on the area of the water surface, volume, and the composition of the habitat. Habitat surface area coupled with the presence of water may provide strong oviposition cues for female mosquitoes. However, volume is likely to fluctuate considerably with rainfall and evaporation altering the quantity of water in any given habitat. At the time of my sampling volume may not have been representative of that in which eggs were originally oviposited. Therefore it may be more useful to look at surface area as a predictor of species occurrence. Sunahara et al. (2002) conducted experiments which showed that surface area plays a significant role in determining oviposition sites for mosquitoes. In my study *Ae. tongae* favoured smaller habitats (surface area < $0.1m^3$) and most likely benefits the species, because habitats of this size often lack aquatic predators (predatory fish). Water boatmen (e.g., *Corixa punctata* IIIiger) and dragonfly larvae (e.g., *Bradinopyga geminata* Rambur, and *Ceriagrion coromandelianum* Fabricius) are known predators of mosquito larvae elsewhere (Quiroz-Martínez and Rodríguez-Castro 2007; Venkatesh and Tyagi 2013) but none were found in any of the habitats sampled. This was despite 16 dragonfly species reported as occurring in Tonga (Marinov 2013), but no nymphs were found co-occurring with mosquito larvae species. Water boatmen were found infrequently in large pools and

ponds after a significant rainfall event. No mosquito larvae were present in these pools at the time of sampling.

Conductivity was also a significant predictor of *Cx. annulirostris* occurrence. Research conducted by Gopalakrishnan et al. (2013) in India found that conductivity in the range 162.9-619 µS cm^{-1} was negatively correlated with larval density of *Aedes* species (*Ae. aegypti* and *Ae. albopictus*). In Gambia, conductivity above 2000 µS cm^{-1} resulted in significant reductions in the larval density of *Anopheles* species (Fillinger et al. 2009). Although larval density was not measured in my study, conductivity measurements between 70-1500 µS cm^{-1} were not negatively correlated with occurrence of *Cx. annulirostris*. These measurements appear abnormally high, but sites in which these measurements were recorded included a rusted 44-gallon drum, old piping, and a car tyre.

A significant volume and temperature interaction was observed for *Ae. aegypti*, *Ae. albopictus,* and *Cx. annulirostris*. As volume increases, the effect of temperature decreases. This suggests that larger, deeper habitats are cooler and would be preferred by these three species. This is not surprising given that larger habitats may be more productive and heterogeneous than smaller habitats (Schoener 1989). It is rare that only one abiotic factor may be at play, and it is likely that combinations of abiotic factors typically determine the presence and abundance of mosquito larvae species (Dunson and Travis 1991). Likewise, a significant volume and conductivity interaction was observed for *Ae. albopictus*. As volume increases, the effect of conductivity decreases. This suggests that larger, deeper habitats have either less organic matter, or there may possibly be ions leaching into habitats like these.

The results of this survey clearly indicate that larval habitats are widespread throughout the Tongatapu Island Group, and that the number of artificial habitats may have significantly increased since previous surveys. The abundance of artificial habitats, particularly car tyres and water containers supports the presence of three of the most common species: *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus. Aedes albopictus* appears to be outcompeting *Ae. aegypti* for habitat and has spread rapidly throughout both Tongatapu and ‘Eua Island. In order to minimise health and

nuisance effects from these mosquitoes, the number of artificial habitats should be reduced. Ways in which habitat reduction can occur will be discussed in the next Chapter.

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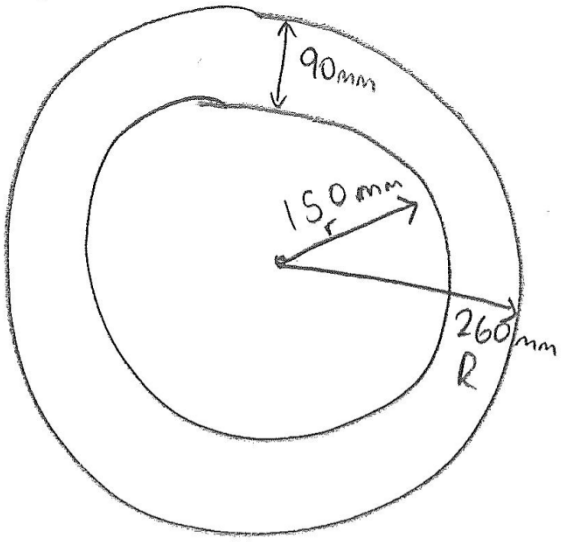
Appendices

Appendix 4.1: Equation and worked exampled shown to calculate the volume of a car tyre.

Car tyre (cut in half) aerial view



$$\text{Area} = \pi R^2 - \pi r^2$$

$$= \pi (R^2 - r^2)$$

$$= 3.14 \times (0.26^2 - 0.15^2)$$

$$= 3.14 \times (0.067 - 0.0225)$$

$$= 3.14 \times 0.045$$

$$\text{Area} = 0.14\ m^2$$

Appendix 4.2: The 23 habitats investigated in the Tongatapu Island Group (n = 84 sites). The number of occurrences, and percentage of occurrence for each habitat is shown (percentages are shown as 1 decimal place).

Habitats	**(n)**	**(%)**	
Car tyre	27	32.1	
Water container (50 Gallon)	7	8.3	
Pond	5	6.0	
Fuel drum (44 Gallon)	5	6.0	
Fridge	4	4.8	
Washing machine	4	4.8	
Bucket	3	3.6	
Cooking pot	3	3.6	
Grass pool	3	3.6	
Plastic container	3	3.6	
Water tank	2	2.4	
Abandoned toilet	2	2.4	
Water drain	2	2.4	
Ice cream container	2	2.4	
Boat	2	2.4	
Tree hole	2	2.4	
Concrete hole	2	2.4	
Wheel rut	1	1.2	
Chilly bin	1	1.2	
Cardboard box	1	1.2	
Wheelbarrow	1	1.2	
Well	1	1.2	
Tarpaulin	1	1.2	
Total	84	100	

Appendix 4.3: Table of species composition and number of occurrences of mosquito species (n = 84 sites). The following species abbreviations were used: “AEG” (*Ae. aegypti*), “ALB” (*Ae. albopictus*), “ANN” (*Cx. annulirostris*), “TON” (*Ae. tongae*), “QUI” (*Cx. quinquefasciatus*), “HOR” (*Ae. horrescens*), “ALB.1” (*Cx. albinervis*), “SIT” (*Cx. sitiens*), “NOC” (*Ae. vexans nocturnus*).

Occurrences	**Species composition**			
1	AEG	ALB	ANN	QUI
23	AEG	ALB		
1	AEG	ALB	SIT	
2	AEG	ALB	QUI	
4	AEG	ALB	TON	
1	AEG	ALB	TON	QUI
7	AEG			
6	AEG	QUI		
3	AEG	TON		
2	AEG	TON	QUI	
1	ALB	ANN	QUI	
8	ALB			
2	ALB	QUI		
2	ALB	TON		
1	HOR			
1	ALB.1	QUI		
3	ANN	QUI		
1	ANN	QUI	SIT	
8	QUI			
1	QUI	SIT		
1	TON			
1	TON	QUI		
4	NOC			

Chapter Five: Synthesis and general discussion

Introduction

The primary aim of my study was to identify mosquito larvae to determine the current mosquito species present in the Tongatapu Island Group and determine species distributions since the last major survey in 2006. Nine species were recorded, of which eight were collected previously by Harding et al. (2007) and one (*Ae. albopictus*) was collected previously by Guillaumot et al. (2012). It is possible that other species occur in the island group, however, as my sampling was spatially extensive any other species are likely to be rare.

I also wished to compare the use of habitats by these species as well as any co-occurrences. The majority of the most common mosquito species collected seemed to colonise any natural and artificial habitat available to them. The three most common mosquito species collected (*Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*) more commonly inhabitated artificial (e.g., car tyres, water containers) over natural habitats, and were widely distributed throughout the Tongatapu Island Group. Reasons for this wide spread distribution is likely a result of the plethora of artificial habitats (particularly car tyres), which occur throughout the Islands. Poor refuse management has likely enabled their spread to locations where mosquitoes may have low chances of colonising without human assistance. The international supply of car tyres around the world has been suggested as the main mechanism of invasion for both *Ae. aegypti* and *Ae. albopictus* globally (Reiter and Sprenger 1987; Benedict et al. 2007). The lack of an island-wide rubbish collection system on Tongatapu has resulted in an abundance of abandoned tyres, which, despite the presence of education programs which inform the population about the dangers of leaving these to fill with water, are available as egg-laying sites by mosquitoes in most towns and villages.

Car tyres are ideal habitats for mosquito larvae as they easily hold rainwater, but are too small to be used by potential aquatic predators. Human-mediated transport of car tyres between islands may well have resulted in the dispersal of *Ae. aegypti* and *Ae. albopictus* into nearby offshore Islands (Pangaimotu, 'Oneata and 'Eua). Assuming that natural mechanisms of dispersal (e.g., wind) are similar for each species, and that each island has suitable habitat available, I would expect that Pangaimotu and 'Oneata islands would have similar species richness to Tongatapu. 'Eua Island may be too far away for easy dispersal by wind. However, if dispersal is mediated by humans (e.g., via boats, airplanes) then one might expect islands with transport hubs (i.e., Tongatapu and 'Eua c.f Pangaimotu and 'Oneata, which each have one small wharf) to have higher species richness. This is in fact the case with Tongatapu and 'Eua having greater species richness than Pangaimotu and 'Oneata. This is not surprising given that there is both a ferry route and an airport on Tongatapu and 'Eua islands, increasing the likelihood of transportation of habitats (car tyres, predominantly) and adult mosquitoes (Figure 5.1). Harding et al. (2007) documented anecdotal evidence of the transport of adult mosquitoes via airplane, and I saw infested car tyres whilst travelling on a boat between Tongatapu and 'Eua Island (Figure 5.2). An international case study has reported the presence of "airport malaria", a term used to describe the recent spread of malaria to Europe and North America due to aircraft carrying infected live mosquitoes from tropical countries (Gratz et al. 2000). Additionally, *Ae. albopictus,* a vector for both dengue fever and chikungunya virus has invaded Europe, North and South America, Asia and numerous countries in the Pacific and Indian Oceans in recent years. Several studies have suggested that these diseases have been mediated via the international used car tyre trade, increased seaborne trade, and global air travel (Tatem et al. 2006; Benedict et al. 2007; Scholte and Schaffner 2007; Paupy et al. 2009; Boukraa et al. 2013).

In Tonga, MacArthur and Wilson's (1967) Island Biogeography theory may be useful in explaining species richness differences between islands. The theory states that the species richness on an island is determined by rates of immigration and extinction. The rate of extinction once a species has colonised an island is affected by island size as larger islands contain more habitats,

enabling greater species richness. Conversely, smaller islands with fewer habitats should have lower species richness. My results are consistent with the predictions of this theory, with nine species recorded on Tongatapu, four on both ‘Eua and ‘Oneata islands and one on Pangaimotu island.

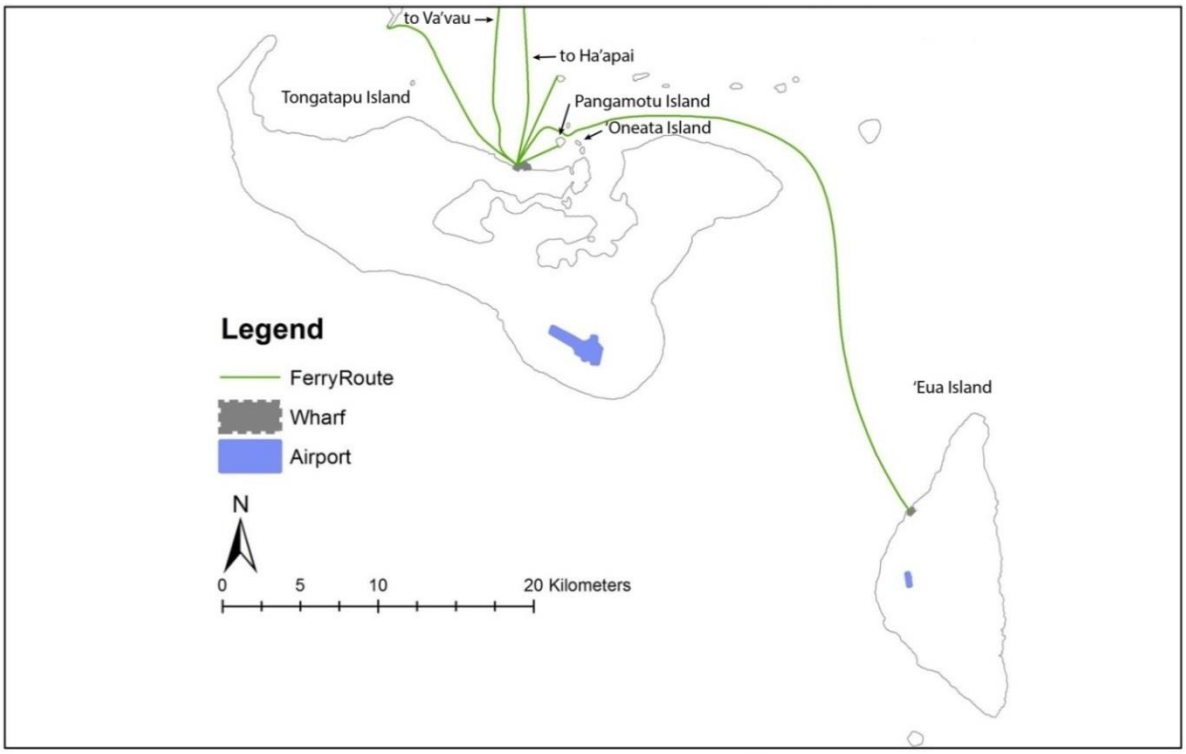


Figure 5.1: Transportation modes from Tongatapu Island to nearby offshore islands in the Tongatapu Island Group.

Figure 5.2: Car tyres being transported from Tongatapu to ‘Eua Island on a boat.

Co-occurrence of species: community assemblage theories

My results presented in Chapter Three and Four showed that co-occurrence of multiple mosquito species frequently happens. However, the drivers of this co-occurrence are not clear. At the core of this question is the fundamental idea of how similar competing species can co-exist in the same habitat if resources are finite. This follows from Gause's principle of competitive exclusion, which states that no two species can remain co-existing if they occupy the same niche (Hardin 1960). In Tonga, four species that commonly co-occur as larvae (*Ae. aegypti*, *Ae. albopictus, Ae. tongae*, *Cx. quinquefasciatus),* appear to require the same food resources and are physiologically similar (Clements 2000). However, the duration of their larval stage is short and resources may not in fact be limiting. Continual recolonisation of habitats via oviposition by adult females further suggests that populations are unlikely to attain and maintain equilibrium populations. Additionally, factors not measured in my study (e.g., the presence of different life history (size) stages of co-occurring species, subtle differences in the use of food resources) may enhance the likelihood of successful species co-occurrence.

For a comprehensive analysis of community assemblages, landscape and ecosystem processes operating at various spatial and temporal scales need to be considered (Turner 1989). This should extend to factors in both terrestrial and aquatic ecosystems, which not only shape communities (e.g., resources availability, presence of predators) but affect the mosquito lifecycle itself. The nature of basal resources (algae, microbial communities) is likely to be important along with other biotic and abiotic factors that drive both terrestrial and aquatic ecosystems (Figure 5.3). An understanding of these factors and the processes that connect them indicate that mosquito lifecycles are complex and influenced by many factors (Figure 5.3). The successful completion of a mosquito's lifecycle is very much influenced by the terrestrial system operating on the aquatic system (e.g., human mediated dispersal and habitat availability).

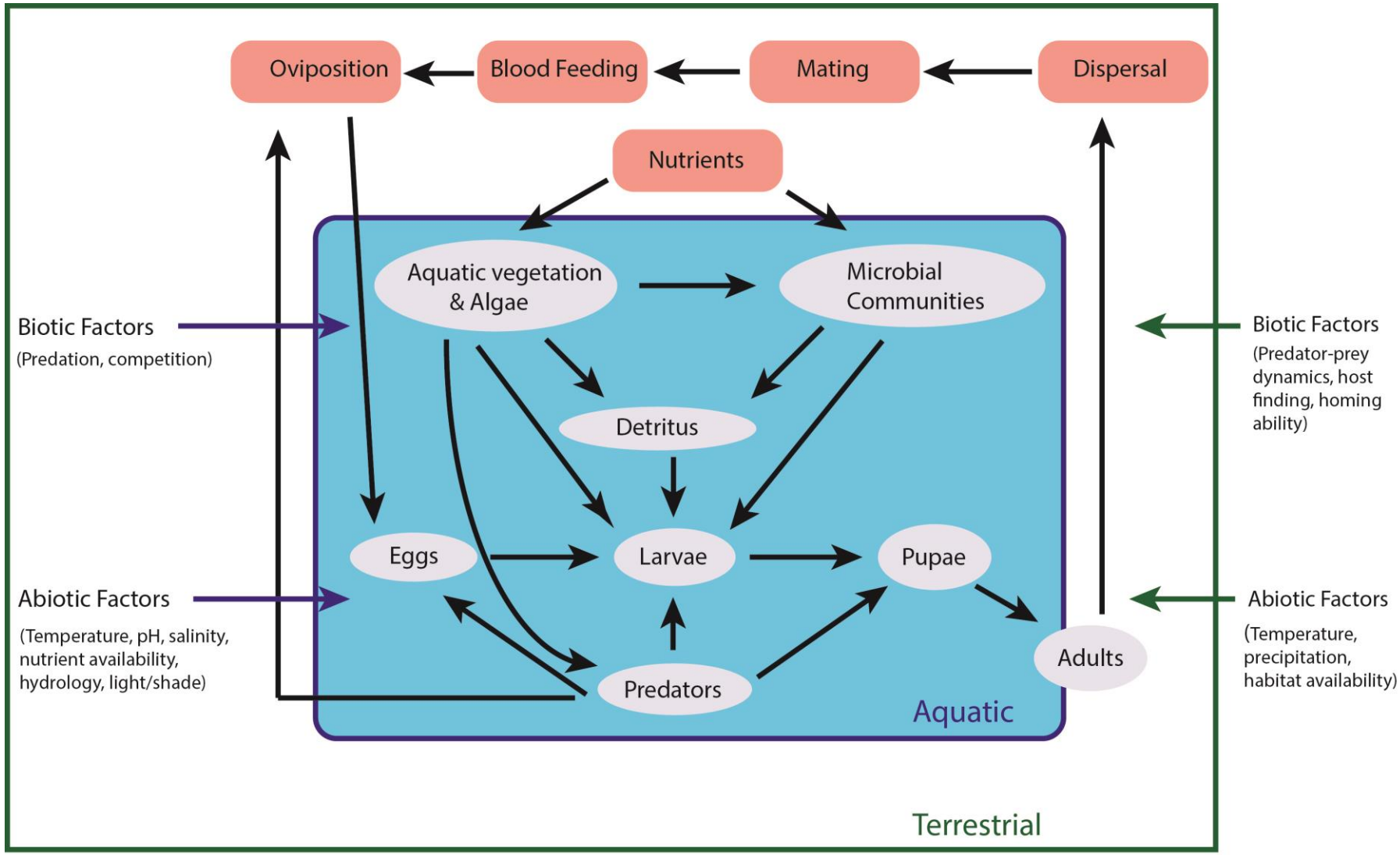


Figure 5.3: Relationships between the mosquito lifecycle and the abiotic and biotic factors which affect it across both terrestrial and aquatic ecosystems. Algae and microbial communities form the basal resources for larval development. Subsequently, adults develop, disperse, mate and feed in the terrestrial environment before ovipositing in an aquatic environment. Each of these aspects of the life cycle is affected by biotic and abiotic factors in both the aquatic (blue arrows) and terrestrial ecosystem (green arrows). Figure modified from Rejmánková et al. 2013.

Identification of mosquito species

Nine mosquito species, five *Aedes* species (*Ae. aegypti*, *Ae. albopictus*, *Ae. horrescens*, *Ae. tongae*, and *Ae. vexans nocturnus*) and four *Culex* species (*Cx. quinquefasciatus*, *Cx. annulirostris*, *Cx. albinervis*, and *Cx. sitiens*) were included in a larval identification key (Chapter Two). A high powered Stereomicroscope (1.6x Leica Plan apochromatic lens) was used to identify mosquito larvae. This was necessary to distinguish between the comb scales of *Ae. aegypti* and *Ae. albopictus*. Genera were first identified visually by observing siphon length. *Culex* species generally had a thinner, longer siphon (siphon length at least three times as long as wide), compared to *Aedes* species in which the siphon was thicker and shorter (siphon length about twice as long as wide). Comb scale morphology was particularly useful for distinguishing *Aedes* species, but for species in which comb scale morphology is identical (*Ae. albopictus* and *Ae. tongae*) the saddle of the anal segment should be inspected. Thus, in *Ae. albopictus* but not in *Ae. tongae* the saddle of the anal segment is incomplete with a colourless, smooth patch ventrally interrupted between its ends for *Ae. albopictus*. Sub-ventral hair tufts located on the siphon of *Culex* species should be examined for distinguishing species.

Mosquito-borne diseases in the Pacific

Very little has been mentioned in this thesis about the epidemiology of mosquito-borne diseases, since the primary focus of my research has been ecological. However, to not mention these diseases would ignore the most important reason for studying mosquito distributions. Mosquitoes kill on average over 700,000 people per year (Gates 2014). In recent years, numerous mosquito-borne disease outbreaks have effected countries throughout the Pacific (Guillaumot 2005; Roth et al. 2014). These diseases have no cure and are primarily spread by *Ae. aegypti* and *Ae. albopictus* (Table 5.1) (Guillaumot et al. 2012; Horwood et al. 2013). Societal issues such as human population density and growth, as well as environmental (e.g, climate) and ecological factors (e.g., wildlife host diversity) are likely to be important factors driving disease dynamics (Jones et al. 2008). Since

January 2012, over 120,000 people throughout the Pacific have been reported to be affected by mosquito-borne diseases, however, this number may be seriously underestimated due to poor reporting (Roth et al. 2014). Underreporting, and a lack of a reporting system, personel, and limited medical information relating to diseases likely exasperates occurrence and transmission of diseases.

A publication by the Secretariat of the Pacific Community (SPC 2014) has produced freely available, real-time, online maps about disease epidemics and occurrences aimed to enhance preparedness for epidemics in respective countries (Figure 5.4).

Table 5.1: Mosquito-borne diseases, symptoms, treatment, hosts and main vectors currently reported in the Pacific. Table modified from Roth et al. (2014).

Disease	**Type**	**Symptoms**	**Treatment**	**Host**	**Main vectors**
Dengue fever	Virus *(Flavivirus)*. 4 serotypes exist (DENV-1, DENV-2, DENV-3, DENV-4).	Rapid onset of intense fever, headaches, joint pains, asthenia, skin rash, nausea, vomitting (Guillaumot 2005).	No antiviral agent exists to treat the infection (Gubler 1998). The disease may alleviate after five to six days, however it can develop into a severe form (dengue haemorrhagic fever) which is fatal without intensive hospitilisation (Guillaumot 2005).	Humans	*Ae. aegypti, Ae.albopictus* (Halstead 2007; World Health Organization 2015).
Dengue haemorrhagic fever (DHF)	Development of dengue fever into a severe form which is fatal without intensive hospitilisation (Guillaumot 2005).	Characterised by a fever whichlasts for 2-7 days. When the fever declines, difficulity breathing, abdominal pain and vomiting may occur. In the next 24–48 hour period, small blood vessels may become excessively permeable and fluid leaking may result in a failure to the circulatory system and shock. This can be fatal and lead to death. (Centers for Disease Control and Prevention 2012).	Primary infection from a DENV serotype may induce lifelong immunity (Halstead 1974; Wilder-Smith et al. 2010)	Humans are thought to be the main host.	*Ae. aegypti, Ae.albopictus* (Gubler 1998).
Chikungunya virus	Virus (Alphavirus)	Fever, skin rash, incapitating joint pain, nausea (World Health Organisation 2014).	No specific treatment available, only treatment directed at relieving symptons (World Health Organisation 2014).	Humans (World Health Organisation, 2014)	*Ae. aegypti, Ae. albopictus* (Gratz 2004).
Zika virus	Virus (Flavivirus). Closely related to dengue fever (Ministry of Health NZ 2014)	Symptons may be closely related to dengue fever. Fever, joint pain, conjunctivitis, headache (Duffy et al. 2009; Hayes 2009).	Diagnosis is firstly by exclusion, based on symptoms and travel history. Symptomatic treatment. No vaccine or preventative drug available (European Centre for Disease Prevention and Control 2015; Ministry of Health NZ 2014).	Originally isolated from rhesus monkey. Transmitted to humans by infected *Aedes* species (Kuno et al. 1998; Simpson 1964).	Numerous *Aedes* speces, including *Ae. aegypti* (Hayes 2009).

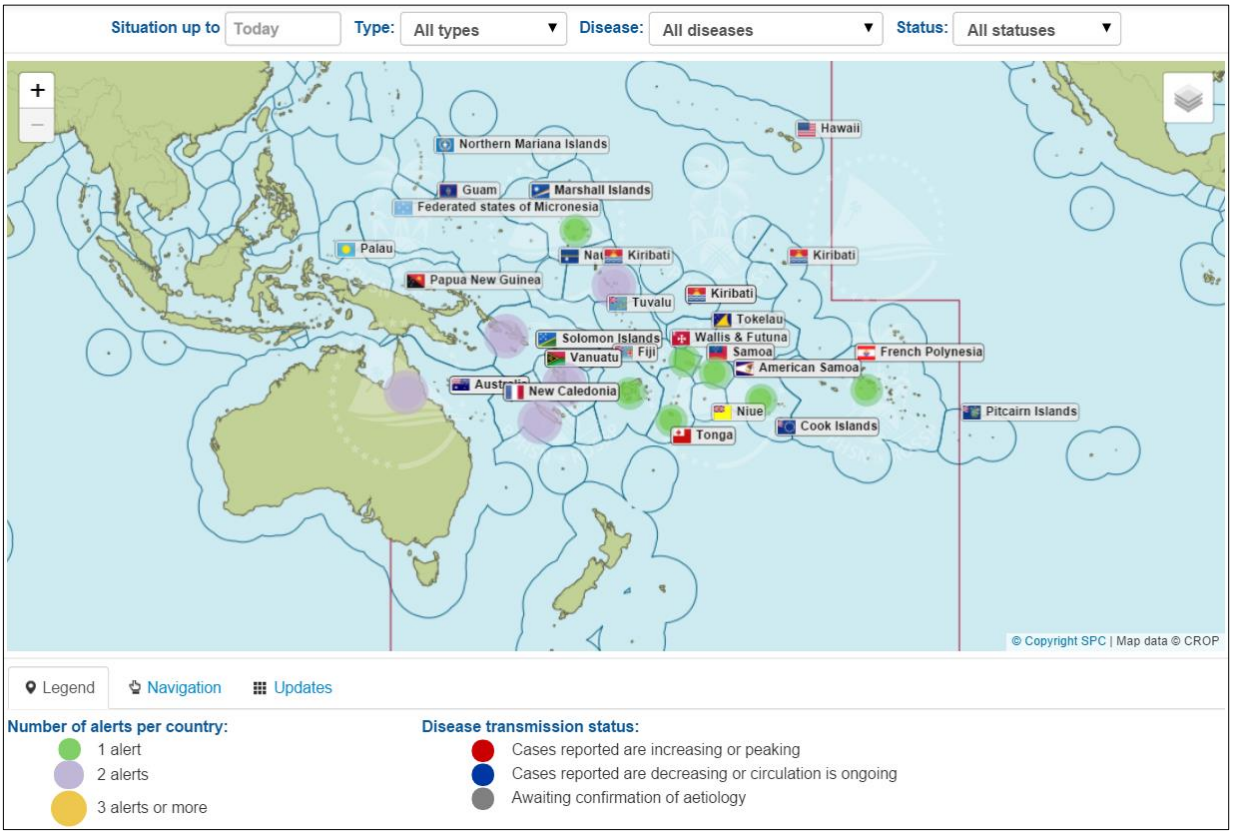


Figure 5.4: Epidemic and emerging disease alerts in the Pacific region as of the June, 4th 2015 (source: SPC, 2014).

Problems, solutions, and management of mosquito threats in Tonga

Poor refuse practices have resulted in a plethora of artificial habitats being available for colonisation by mosquitoes. The removal of such habitats can greatly reduce the likelihood of mosquitoes ovipositing in an area (Richards et al. 2008). On-going community based education programmes involving school students, church groups, and citizen scientists should be adopted to educate and reduce anthropogenic derived habitats. Community participation is an efficient and cost-effective means of reducing mosquitoes, but requires community ownership, resources, ideas, organisation, and leadership to achieve long-term success (Gubler and Clark 1996). Without the involvement of community leaders, local government projects are less likely to be successful (Winch et al. 1992). Furthermore, a key emphasis should be on programs planned, directed and implemented by community members (bottom-up), rather than by government officials and aid agencies (top-down).

Thus, it is well-known that programmes setup by government officials and aid organisations alone are less likely to be successful, due to the capacity for these programmes to be maintained in the long-term by communities who were not involved in the initial planning process (Gubler and Clark 1996). One example is a case in Thailand (Phanthumachinda et al. 1985), in which programmes were initiated by government officials with support from the World Health Organization (WHO) to reduce the incidence of dengue fever through source reduction (the removal of habitat used by mosquito larvae). Members of the community participated but were not involved in the planning, implementation and direction of the project, so when support was withdrawn from aid agencies, so too was community involvement and maintenance of the project.

Community-based education approaches have also been implemented internationally. For instance, efforts to reduce dengue hemorrhagic fever outbreaks in Indonesia involved the training of 500 community members to educate the public (Soedarmo 1994). Similarly, members of the community in Singapore were educated by 1,500 government inspectors who acted as health educators and law enforcement officers. This program focused on source reduction by making it illegal for citizens to have potential mosquito larvae habitats on their properties. The program was highly successful with a house index (percentage of houses infested with larvae or pupae) of less than 5% throughout most of Singapore. It was also highly motivational, as not participating in the programme meant breaking the law which resulted in economic ramifications. Fines totalling approximately $US800,000 were gathered by the government for breaking this law (Chan et al. 1990), which was used for further source reduction operations and education.

In Tonga, a combination of community-based approaches (bottom-up) and government involvement (top-down) is recommended. The community should drive the project, designing and implementing practices, whilst government officials oversee the concept and rigour of the project. A project should exist which focuses on source reduction, either through rubbish clean-ups, or by tipping over habitats to remove water, and then covering them up. Water is not easily removed from

car tyres because of their shape. Consequently, car tyres should be filled with soil and perhaps used for plantings. In fact this procedure was followed in Tonga in 2007 following recommendations by Harding et al. (2007), but was not continued in subsequent years (Jon Harding, pers. comm., 3rd June 2015). Car tyre vendors operating in Tongatapu should also cover their car tyres with tarpaulin to avoid mosquito larvae infestations, which have been found in most tyres (Figure 5.5). Covering other artificial habitats with cloths or fine wire-mesh will also prevent egg-laying by mosquitoes.

Figure 5.5: Car tyre vendors in Tongatapu. (a) near Vaiola hospital, (b) in Nuku'alofa city centre.

Community leaders should take charge of educating village people and put responsibility on property owners to check for larval habitats. Incurring economic ramifications for not disposing of larval habitats, as has been done in Singapore (Chan et al. 1990) is not practical in Tonga. However, incentives and rewards for villages which are monitoring and disposing of larval habitats should be incorporated in the project. In the case of a disease outbreak, adult mosquitoes should be targeted through pesticide spraying in the village (and nearby villages) of origin. Larval habitats should then be searched and disposed of by community members after pesticide has been used to reduce chances of recolonisation. The Tongan Ministry of Health (MOH) already does this (Tonga Ministry of Health, pers. comm. 5th December 2013), but habitat disposal after spraying should be the responsibility of village members. Additionally, *Spinosad* a derivative of the bacterium *Saccharopolyspora spinosa* can act as a mosquito larvicide (Kirst et al. 1992). It has been used

successfully as a larvicide in used car tyres in Mexico, where a significant reduction in numbers of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* was obtained (Marina et al. 2012). *Spinosad* can be effective for up to 4–6 weeks after first application (Garza-Robledo et al. 2011) and the spraying of used car tyres with it should be considered if soil infilling is not adopted by community members.

On several occasions, Tongan families visited in this study either did not know, or did not care that mosquito larvae were occurring in habitats around their properties. It quickly became apparent that many Tongan people lacked knowledge that mosquito larvae metamorphosed into potential disease vectors as adult mosquitoes, and in many cases, people mistook mosquito larvae for harmless aquatic worms. This was alarming to hear, and the forefront of any campaign in the future should begin with education in schools and communities to ensure that everybody understands what a mosquito larvae is, and why it is important to reduce larval habitats.

The pragmatic solutions proposed above are cost-effective and straightforward. The combination of education and project development through community-based approaches should result in a significant reduction of larval habitats and subsequently adult mosquitoes and disease.

Limitations of my work and suggestions for future research

Due to the difficulty of collecting mosquito larvae in some habitats (e.g., accessibility and avoidance behaviour of larvae), the time and effort (number of dips used to acquire mosquito larvae) spent collecting in each habitat differed. For this reason, I only used presence/absence or binomial data, as opposed to abundance data. Abundance data would have enabled greater understanding of habitat associations and drivers of community assembly. Of particular interest, it would have been possible to test the abundances of both *Ae. albopictus* and *Ae. aegypti* in different habitat types, and where they co-occurred. Additionally, when sampling habitats I searched for mosquitoes and actively "dipped" and chased larvae in order to collect as many larvae as possible. However, I may have missed some larvae due to their avoidance behaviour as larvae commonly swim to the bottom of a habitat when a shadow or disturbance in the water column occurs. Therefore, I suggest that a strict

methodology be developed with respect to the number of "dips" used in a particular habitat, where in a habitat dips are made, and the amount of time spent at each habitat.

Secondly, I sampled many more artificial habitats (n=74) than natural habitats (n=10). Artificial habitats were predominate over natural habitats in most villages, so it was impossible to sample even numbers of natural and artificial habitats. In fact, in most villages where I sampled no natural habitats were present. This likely biased results favouring collection of species that inhabit artificial habitats, but it reflected the reality of the situation. Therefore, it would be interesting to do further sampling over multiple seasons (wet and dry seasons) to see whether the ratio of artificial-natural habitats changes and whether the larval distributional patterns of species change if more natural pools are present.

Thirdly, a number of indices commonly used to estimate larval populations within a community were not measured in this study. For example, the Breteau index (the number of water-filled containers containing larvae and/or pupae per 100 homes), the house index (percentage of houses infested with larvae or pupae) and the container index (percentage of water-holding containers infested with larvae or pupae) are commonly measured in mosquito survey studies (Focks 2003). These three indices would allow comparisons to be made between different survey seasons in subsequent years.

Future researchers also need to be aware of language barriers which may exist. For example, when I visited villages and asked about larval habitats, many people were confused because they did not know or understand what mosquito larvae were. The translation of "mosquito larvae" to "Ikeika'âvai" in Tongan significantly increased understanding and resulted in more effective communication. Asking people if they had seen any "Ikeika'âvai" was more effective and should be used if communication appears to be an issue.

Additionally, future research should focus on conducting a mosquito survey of Ha'apai and Va'vau to re-assess the mosquito distribution since the survey of Harding et al. (2007). Of particular interest will be whether *Ae. albopictus* has colonised these islands. The Niue Islands (located near Va'vau) would also be interesting to survey, as very little is known about which mosquito species occur there. Shipping routes from Va'vau to Niue likely transport used car tyres which may have enabled mosquitoes to colonise there.

Overall conclusion

Nine mosquito species were collected in a survey of the Tongatapu Island Group in 2013, compared to eight species collected in a survey in 2006. *Aedes aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* were the most commonly collected species. Artificial habitats were preferred by these species, with car tyres being their most common larval habitat. Finding the widespread distribution and occurrence of *Ae. albopictus* in Tongatapu was the most striking outcome of this survey, as it was first identified in Tonga in 2012 (Guillaumot et al. 2012). Reduction of larval habitats through refuse disposal, educating inhabitants to cover and tip out water, and the infilling of habitats with soil and plantings should greatly reduce the presence of larvae and adult mosquitoes throughout the Tongatapu Island Group. The pictorial identification key to larvae included in this thesis will be a useful resource to aid future workers to correctly identify species currently found in the Tongatapu Island Group.

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